CIGARETTE SMOKE, BLACK RASPBERRIES, AND ORAL CANCER PREVENTION

AN EXPLORATORY STUDY

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CHAPTER ONE: INTRODUCTION AND BACKGROUND

INTRODUCTION

Oral cancer is a growing health problem worldwide, with over 300,000 new cases expected annually. In the U.S. alone, nearly 50,000 new cases are expected in 2017 (American Cancer Society, 2016). Complex interactions between environmental exposures, behavioral, and genetic factors increase risk for the disease; the major risk factors are tobacco use, alcohol consumption, human papilloma virus (HPV), and genetics. It was estimated in 2005 that tobacco use accounted for 90% of oral cancers globally (WHO, 2005). There are 1,200,000,000 tobacco users worldwide, and nearly 6.8 trillion cigarettes smoked per year (Lung Foundation, 2015). While many developed countries have experienced a decline in recent years, smoking rates continue to rise in densely populated parts of the developing world (Lung Foundation, 2015). As overall smoking rates rise, the global burden of tobacco-related cancers, including oral cancer, continues to increase. Current research is exploring ways to minimize this burden through prevention methods that go beyond tobacco control and smoking cessation. Studies have demonstrated a significant correlation between increased consumption of fruits and vegetables and decreased oral cancer risk (Steinmetz and Potter, 1996). Specifically, the chemical compounds or "phytochemicals" in black raspberries (BRBs) have been shown to decrease the expression of cancer-associated genes in humans (Knobloch et al., 2016). The current study investigates the impact of BRB treatment and cigarette smoke (CS) exposure on human oral cell lines in order to assess the potential of BRBs to prevent oral cancer in people at high risk due to smoking.

ORAL CANCER

Development

Environmental exposures over time cause an accumulation of DNA damage that results in the development of malignancies in the mouth. DNA damage leads to unregulated cell growth, which drives a stepwise evolution first from normal tissue to premalignant high at-risk mucosa (HARM), then finally from HARM to clearly malignant tissue. In other words, oral cancer occurs in a multi-step process (Farber, 1984, Califano, 1996).

It was first postulated in 1953 that exposure to carcinogens puts the entire oral mucosal field at risk of developing malignancies. After observing cancers reoccur in apparently normal tissue following the surgical resection of cancerous lesions, Danley Slaughter coined the term "field cancerization." (Slaughter, 1953) These biological processes tell us two things about the damage caused by smoking: First, it happens slowly over time in a stepwise fashion, offering plenty of opportunity to intervene. Second, it puts the entire field, including all apparently normal tissue, at risk. Just one microscopically visible malignancy or pre-malignancy indicates the entire oral cavity is susceptible to cancer.

Incidence

Oral cancer incidence rates have continued to increase in the U.S, especially among men (American Cancer Society, 2016). Both globally and domestically, there is a notable gender disparity. In the U.S. there are almost three male cases for every one female case. This trend has been attributed to behavioral risk factors, like

tobacco use and alcohol consumption, and rising rates of HPV-related cancers in men. Estimated new cases and deaths in males and females can be found in **Table 1**.

Outcomes

Cases are usually treated with a combination of surgical resection and chemo- or radiation therapy. These methods are costly and often greatly compromise quality of life. There is also a high likelihood of reoccurrence – malignant lesions reappear in 20% of patients just 18 months following surgical resection (Cooper et al. 1989). This contributes to dreadful 5- and 10-year survival rates, at 63% and 52%, respectively. When diagnosis occurs at a local stage, the 5-year survival rate jumps to 83%, but less than one-third of cases are detected at this time (American Cancer Society, 2016).

CIGARETTE SMOKE EXPOSURE

Cigarette smoking is the most common cause of oral cancer. The Centers for Disease Control and Prevention (CDC), the nation's health protection agency, reported that smoking accounts for 1 in 5 deaths in the U.S., making it the leading cause of preventable disease and death. The 60+ carcinogens in cigarette smoke increase the risk of numerous types of cancers, including those of the lung, larynx, stomach, esophagus, liver, pancreas, and oral cavity (Hecht, 2003). Carcinogens in cigarette smoke can bind to DNA, forming DNA adducts (DNA bound to a cancer-causing chemical). Normal cells with intact repair mechanisms are able to eliminate these adducts and restore DNA to its normal state. However, continuous cigarette smoke exposure over a long period of time increases the likelihood that an adduct will escape repair and cause permanent mutations (Hecht, 2003). This is a crucial step in the development of cancer. Studies have established that cigarette smoke carcinogens eventually cause miscoding, or erroneous base-pairing, during DNA replication. When these mutations afflict important cancer-causing genes, they can disturb cellular growth-control mechanisms, leading to uncontrolled cellular proliferation (Loechler and Green, 1984, Singer and Essigmann, 1991, Seo et al., 2000). In this way, the multi-step process leading to cancer is initiated.

As of 2015, 36.5 million adults in the U.S. smoked cigarettes, and 16 million Americans were living with a smoking-related diseases (National Institute of Dental and Craniofacial Research). Like many health behaviors, the prevalence of smoking varies widely between social groups, especially with regard to socioeconomic status. The current smoking rate above the federal poverty level is 14%, but it jumps to 26% in people living at or below the federal poverty line (American Cancer Society, 2016). The discrepancy is even more pronounced when smoking rates are stratified by education. Only 4% of people with graduate degrees smoke. But for people with GED certificates, the current smoking rate is 34% (American Cancer Society, 2016). Although the current smoking rate continues to decline nationwide, certain groups are more at risk than ever.

FRUITS, VEGETABLES, AND (ORAL) CANCER: A BRIEF REVIEW OF THE EVIDENCE.

In 1992, a review of 200 studies concluded that fruit and vegetable consumption is inversely associated with cancer incidence. 128 of 156 dietary studies examining the relationship between fruit and vegetable intake and various cancers found a statistically significant protective effect (Block, 1992). It is specified that high intake of fruits and vegetables was particularly effective in decreasing risk for cancers of the esophagus, oral

cavity and larynx. In a 1998 study of oral cancer, a doubling of oral cancer risk was observed among people in the lower quartile of fruit consumption (vs. the highest quartile) (McLaughlin et al., 1988). A review published in 1996 concluded that data consistently supported a halving of risk between high versus low intake of fruit, and even postulated that the strength of this association is underestimated due to the methodology of nutritional epidemiological studies that must adjust for random subject error and other factors (Steinmetz and Potter, 1996).

However, recent studies have come to more ambiguous conclusions about the power of produce. In 2003, the International Agency for Research on Cancer classified the evidence as "limited." A British mini-review in 2011 determined that the correlation has not been firmly established for any cancers besides those of the upper GI tract, including the oral cavity (Cooper et al., 1989). They acknowledged that an inverse relationship between oral cancer incidence and fruit and vegetable consumption continues to be supported, although the observed reduction in risk was much smaller than had been predicted in the past. A large European epidemiological study from 1992-2000 with nearly 500,000 participants observed only a very small inverse association between produce intake and cancer incidence (HR=0.97). But in cancers linked to smoking or alcohol drinking, the protective effect was more pronounced (Boffetta et al., 2010).

This discrepancy may be because previous studies reporting a strong correlation were case-control studies, whereas more recent studies have shifted to epidemiological methods that rely on self-reporting in food frequency questionnaires. The inaccuracy of diet self-reporting is well established. If most people were unable to accurately report what they ate, then study results were likely affected. Nevertheless, even studies yielding the most conservative estimates of the correlation between cancer and fruit and vegetable intake maintain that it is significantly correlated with cancers of the upper aerodigestive tract.

DIETARY WHOLE BRBS AS A METHOD OF CHEMOPREVENTION FOR ORAL CANCER

Chemoprevention employs natural or synthetic chemicals to prevent, suppress, or reverse cancer. BRBs in their lyophilized (freeze-dried) form have been shown to prevent various cancers and to decrease expression of cancer-associated genes in human cell culture, animal models, and clinical trials. BRBs contain many compounds with known chemopreventive activity, including anthocyanins, vitamins C and E, calcium, folic acid, and ellagic acid (Sreeram and Stoner, 2011). One animal model tested the effectiveness of topical BRBs in inhibiting the progression of premalignant lesions similar to the HARM environment found in smokers. After being exposed to chemical carcinogen, hamsters were treated topically with BRBs 3 days a week for either 2 or 6 weeks. It was found that the longer-term treatment lowered incidence of new lesions, prevented existing lesions from multiplying, and decreased cancerous cell proliferation. Both the shorter-term and longer-term conditions altered expression of molecular biomarkers associated with oral cancer (Warner et al., 2014).

In clinical studies on humans, BRBs have been shown to have positive effects on premalignant lesions or cancers of the oral cavity, esophagus and colon (Kresty et al., 2016). A phase 0 study testing whether BRB troches could reduce pro-inflammatory and anti-apoptotic gene expression in pre-surgical oral cancer patients showed that the troche did indeed significantly reduce expression of pro-survival and pro-inflammatory genes

in oral tissues (Knobloch et al., 2010). In one study, people with microscopically confirmed oral high at-risk mucosa (HARM) premalignant tissue applied 10% BRB gel (0.5 g BRB) 4 times daily for 6 weeks. Results were promising, with 41% of patients showing decreased lesional grade and 23% an increased histologic grade (Shumway et al., 2008)

Another placebo controlled clinical trial treated 40 patients with confirmed oral premalignancies with BRB gel for 3 months to test efficacy. In the treatment group, topical application of BRB gel resulted in significant reductions in lesion size, histologic grade and loss of heterozygosity (LOH) events. In fact, 76% of BRB treated lesions decreased in size, whereas 71% of placebo treated lesions increased in size (Mallery et al., 2014). These are just a few examples illustrating the clinical effectiveness of BRBs as a chemopreventive agent.

CHAPTER TWO: CELL CULTURE METHODS

Cell culture *in vitro* methods leverage the ability of dispersed cells to grow in an artificial two-dimensional model system surrounded by essential nutrients and growth factors. Some cells, such as oral epithelial cells, require a solid surface for attachment as well as an optimized environmental space regulated for temperature, atmospheric composition, and humidity. These in vitro systems allow a great deal of control over the manipulation of user-directed variables to provide detailed information on cellular biochemical/biomolecular responses.

CELL LINE INVENTORY

TE-1177 (Normal Phenotype): This is a normal, human oral epithelial cell line derived from tonsil epithelium. It has a finite lifespan, undergoes senescence, and does not grow tumors when xenotransplanted into athymic "nude" mouse models. Unlike non-normal phenotypes, its growth is anchorage-dependent and contact-inhibited – the cells must be attached to a solid surface in order to grow, and they stop proliferating once the plate is fully confluent.

SCC-83-01-82 ("SCC-83" or HARM Premalignant Phenotype): This is a non-tumorigenic, premalignant human squamous cell carcinoma cell line. It was derived from a human oral tumor but does not form tumors when grown in nude mice. Unlike normal cell lines, it is immortalized (divides indefinitely) and capable of anchorage-independent growth.27 In this paper, this cell line is referred to as SCC-83 or high at-risk mucosal (HARM) oral cells.

SCC-83-01-82-CA ("CA-83" or Malignant Phenotype): This is a tumorigenic, malignant squamous cell carcinoma cell line derived from the premalignant human cell line described above. In order to obtain the aggressively tumorigenic CA-83 cells, the chemical mutagen methyl methanesulfonate (MMS) was used to convert SCC-83 cells to a malignant phenotype. Unlike their untreated counterparts, MMS-treated SCC-83 can produce tumors in mice within 3–5 months. Like SCC-83 cells, it is immortalized and capable of anchorage-independent growth (Milo et al., 1990). Comparisons between transcriptional changes in these two cell lines are especially intriguing due to their shared genetic background – they allow for more certainty that the results are meaningful, rather than just a function of different genomes. **Table 2.** Compares characteristics of these three cell lines.

EPITHELIAL CELL GROWTH MEDIA AND CONDITIONS

Human normal (TE-1177), HARM premalignant (SCC-83), and malignant (CA-83) oral cells were grown in Dulbecco's Modified Eagle's Medium (D-MEM) supplemented with 10% fetal bovine serum (FBS). Cells were allowed to grow in a controlled environmental chamber at 37°C in 5% CO2 with 90% relative humidity. D-MEM is a liquid growth medium containing essential amino acids, vitamins, inorganic salts, and glucose. D-MEM also contains the pH indicator dye phenol red, which is able to follow colorimetric shifts between acidic pH 6 (yellow) and basic pH8 (purple).

BRB TREATMENT AND CS EXPOSURE SCHEMAS

Each cell line generated 24 biological samples through the independent exposure with either cigarette smoke extract (CS-E, % v/v) or treatment with black raspberry extract (BRB-E, µg/mL) in a dose-escalation format. In summary, each cell line required two 6-well plates per experimental condition (CS-E or BRB-E) with a total of 72 *in vitro* samples generated. See **Figure 1** for the full experimental plate set up.

BLACK RASPBERRY EXTRACT TREATMENT OF TE-1177, SCC-83, AND CA-83 CELLS

GENERATION OF BLACK RASPBERRY EXTRACT (BRB-E)

The black raspberries (*Rubus occidentalis 'Jewel' variety*) used in this study were grown at the Dale Stokes Raspberry Farm in Wilmington, OH. All BRBs were grown in the same area and picked at the same degree of ripeness. A dedicated lot was harvested, washed, and frozen at -20°C. They were transported frozen to Van Drunen Farms, where they were lyophilized (freeze-dried) and ground into a fine powder. An ethanol/water (4:1) solution was used to extract freeze-dried BRB powder to create an "extract" enriched for polar hydrophilic (water-loving) phytochemicals, including the anthocyaninic compounds that give ripe BRBs their deep purple color. The BRB-E was dried under nitrogen to remove any traces of the ethanol used in the extraction process, leaving a dark purple solid crystalline powder. To make BRB-E usable for *in vitro* studies, the crystalline extract was resuspended in dimethyl sulfoxide (DMSO) as a 1000× Stock Solution at a concentration of 200 mg/mL. DMSO was used as a delivery vehicle, instead of water for instance, was used since the organic nature of DMSO allows for phytochemicals that are not strongly water-soluble to go back into solution. Also, the levels of DMSO used in these studies do not have a significant biological effect on the growth of the cells. The BRB-E was stored at -86°C until needed for cell culture assays (Sreeram and Stoner, 2011).

EXPERIMENTAL DESIGN

Normal, HARM premalignant, and malignant oral epithelial cells were plated in 6-well plates with 2 mL of growth media per well. Each well was treated for 48 hours following a dose escalation format, such that 100, 200, 400, and 800 µg/mL doses of BRB-E or 200 and 800 µg/mL doses of DMSO vehicle. For each cell line there were two replicates of six different treatments, making a total of 12 samples per cell line (See **Figure 1**).

CIGARETTE SMOKE EXTRACT EXPOSURE TO TE-1177, SCC-83, AND CA-83 CELLS

GENERATION OF CIGARETTE SMOKE EXTRACT (CS-E)

Cigarette smoke extract was obtained by aspirating four University of Kentucky 1R6F Reference Cigarettes (**Figure 2**) through 20 mL of DMEM containing 0.5% FBS under constant vacuum using a custom assembled smoking apparatus (**Figures 3-4**). The CS-E was generated fresh or stored at -86°C in 2 mL aliquots for less than

a week. Stored CS-E aliquots were thawed immediately prior to use during *in vitro* experiments. A review of literature was conducted prior to construction of the custom smoking apparatus in order to gain an understanding of common methods used to conduct *in vitro* cigarette smoking assays using non-commercial smoking equipment. **Table 3** summarizes the results of the review, which included eleven papers published between 1996 and 2016.

There were some important constants revealed during this literature review—for example, almost every publication reported sterilizing the extract prior to treatment. In general, there were a large variety of methods used. **Figures 3-4** illustrate the smoking device that was constructed after considerable trial and error and ultimately used for these experiments. The reference cigarette was held by a modified syringe inserted into a 10 mL serological pipet. Smoke was aspirated directly into the bottom of a 50 mL polypropylene tube containing 20 mL of cell culture media. Combustion compounds and particulate matter from the percolating cigarette smoke consequently become incorporated into the culture medium matrix. The excess pass-through smoke was subsequently drawn through two in-series Erlenmeyer flask water traps to reduce the opportunity for it to be drawn into the house vacuum system.

CS-*E IN VITRO* TREATMENT OF ORAL CELL LINES

Normal (TE-1177), HARM premalignant (SCC-83) and malignant (CA-83) oral epithelial cells were plated in 6-well plates with 2 mL of growth media per well. Each well was exposed for 48 hours in a dose escalation format, with 0, 1.25, 2.5, 5, 10 and 20% volume/volume CS-E doses, i.e. 10% CS-E indicates 0.2 mL of CS-E per 2 mL D-MEM. In each cell line, there were two biological replicates of each of the six different experimental exposures, making a total of 12 CS-E samples per cell line in addition to the 12 BRB-E samples (See **Figure 1**.)

TOTAL RNA PURIFICATION

After 48 hours of exposure or treatment, growth media was aspirated and cells were rinsed with phosphate-buffered saline. Qiagen Buffer RLT (0.6 mL) supplemented with 0.01% β-mercaptoethanol was added to each well to lyse the cells in place without further manipulation. The lysate for each sample was collected into a prelabeled 2 mL microcentrifuge tube and stored at -86°C pending further processing. Total RNA was purified using the Qiagen RNeasy Mini Kit (Valencia, CA) and a Qiagen QIAvac 24 multiport vacuum manifold. All biological waste and chemical flow-through was collected in the base of the manifold and discarded appropriately. In brief:

- The RLT Buffer lysed cells were homogenized using a Qiagen QIAshredder spin column centrifuged for 2 minutes at full speed (>14,000 *g*).
- One volume (~0.6 mL) of 70% ethanol was added to the homogenized lysate, and these samples were transferred to RNeasy spin columns on a vacuum manifold. RNeasy columns are affinity chromatography columns that selectively bind RNA under the different conditions created by the isolation solutions.
- The vacuum was switched on and applied until the lysate solution completely passed through the RNeasy column.

- Buffer RW1 was added to each RNeasy column. The vacuum was switched on until the buffer flow-through was complete. This helped remove contaminating DNA from the RNeasy columns.
- Buffer RPE was added to each RNeasy column. The vacuum was switched on until the buffer flow-through was complete. This step was repeated to assist in removing all non-RNA molecules, such as proteins, from the RNeasy column.
- RNeasy columns were each placed in a 2 mL collection tube and centrifuged at full speed (>14,000 *g*) for 1 minute. Any residual flow-through from this step was discarded. The RNeasy column should now contain purified total RNA.
- Each RNeasy column was placed in another new collection tube, and RNase-free water was applied directly to each spin column surface. RNA does not bind to the RNeasy column in the absence of salts, such that pure water pulls the RNA off the column.
- The RNeasy columns were centrifuged for 1 minute at full speed (>14,000 *g*) to elute the RNA into the collection tube.
- The purified total RNA was stored at -86°C until needed for analysis.

RNA NORMALIZATION

After purification, total RNA concentration was determined using the Thermo Scientific NanoDrop microvolume UV-Vis spectrophotometer. Total RNA was diluted with RNase-free water and normalized to uniform concentrations (ng/µL) within cell lines and treatments. This allows fixed volumes to be used when designing subsequent master mixes of chemistry, and decreases the chances of pipetting errors. See **Table i** (Appendix) for representative RNA normalizations and dilutions.

FIRST STRAND CDNA SYNTHESIS

First strand synthesis is necessary to convert single stranded RNA molecules into single stranded complementary DNA (cDNA) molecules that can be used for polymerase chain reaction (PCR) assays. Only single stranded DNA can be used as the starting template material to amplify more DNA during PCR. The "second" strand synthesis reaction occurs during the PCR amplification process. Applied Biosystems cDNA Reverse Transcription Kit was used according to the manufacturer's protocol for first strand cDNA synthesis. RNA volumes used were adjusted as necessary according to concentrations so that each cDNA synthesis reaction was performed with 100 ng of RNA. The recommended range for cDNA synthesis is 1–1000 ng of total RNA.

CHAPTER THREE: FROM BEDSIDE TO BENCH: VALIDATION OF CIGARETTE SMOKE BIOMARKERS IN NORMAL AND HIGH AT-RISK MUCOSA (HARM) ORAL CELLS

OBJECTIVE

In a previous dietary intervention clinical trial (NCT01514552, clinicaltrials.gov) with smokers and non-smokers, we validated several cigarette smoke (CS)-associated transcriptional biomarkers present in the mRNA obtained from non-invasive oral brush biopsies. Transcriptional biomarkers are genes that exhibit differential changes in expression in response to an environmental exposure, such as CS. This study used the previously described *in vitro* experiments to investigate changes in gene expression in both normal and HARM premalignant oral cells following exposure to CS or administration of BRB phytochemicals in order to validate a clinical signature *in vitro*, and search for pathways through which BRBs may prevent oral cancer development in smokers. The genes assessed in this study, along with the proteins they encode, are displayed in **Table 4**.

The study from which these genes were obtained was a phase I clinical trial that investigated the effects of strawberry gummy consumption on oral tissue in 36 smoking and non-smoking adults. Food scientists at OSU developed a strawberry gummy designed to dissolve slowly in the mouth. Smokers and non-smokers were each divided into treatment and placebo groups. Over the course of the study, blood, urine, saliva, and oral brush biopsies were used to assess the physiological effect of the strawberry gummy. The oral brush biopsies revealed genes with differential expression in oral tissue between the smoking and non-smoking groups, and 8 of these genes were assessed in this experiment.

REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION (RT-qPCR) METHODS

Real-time quantitative polymerase chain reactions were used to assess changes in expression of eight biomarkers of cigarette smoke exposure. 48 samples were studied: 24 normal and 24 HARM, with each group containing 12 CS-E-exposed and 12 BRB-E-treated samples. Each 48-well PCR plate (pictured in **Figure 5**) tested all eight genes in six samples – in other words, each PCR plate corresponded to a single six-well cell culture plate. There were eight unique PCR plates done in replicate for a total of 16 plates and 768 reactions, not including the negative controls.

ALOX12B, *CD207*, *HTR3A*, *KRT10*, and *PNLRP1* were excluded from this study because they failed to amplify consistently. Later tests on RNA samples from human oral tissue and HeLa cells (an immortal cell line derived from cancerous cervical cells) proved that these five gene assays were nonfunctional, likely due to mishandling. Of the three remaining genes that amplified consistently, GeneNorm software selected *DUSP1* as a reference gene, because it exhibited the least variation in expression. *ACTB* and *TRNP1* expression was normalized to the reference using qbase+ software.

RESULTS

See **Tables 5-6** for fold-changes in gene expression arising from each treatment. BRB-E treatment induced no significant changes in expression in the two biomarkers in either of the cell lines. BRBs may be able to reverse transcriptional changes caused by CS-E exposure, but it did not alter these two specific biomarkers in this study. Likewise, CS-E did not affect expression of the two genes in normal cells. While this was initially a surprise, it makes sense that CS did not cause lasting change to normal gene expression, because normal repair mechanisms are still intact. However, CS significantly altered both *ACTB* and *TRNP1* in HARM cells. The change in *ACTB*, while small, was statistically significant and unexpected – the gene is historically used as a reference. However, *ACTB* has been previously reported to be involved in the regulation of normal cell cycle progression (Sun et. al, 2012).

Excitingly, *TRNP1* showed an 8-fold increase in expression, validating it as a biomarker of CS exposure *in vitro*. While a few studies have examined its role, there is still much to learn about the role of *TRNP1*. A study in mice concluded that it regulates expansion and gyrification (folding) of the mammalian cerebral cortex (Stahl et al., 2013). Another states that the protein it encodes, TMF-regulated nuclear protein, accelerates cell-cycle progression (Volpe, 2006). Because it has now been validated both *in vivo* and *in vitro* as a biomarker responsive to CS-exposure, studies should seek to better understand its function, including any role it might play in carcinogenesis.

CHAPTER FOUR: EXPLORATORY STUDIES INTERROGATING CS- AND BRB-RESPONSIVE GENES USING CANCER PATHWAY-FOCUSED PCR EXPRESSION ARRAYS

OBJECTIVE

This study used the previously described *in vitro* experiments to investigate changes in gene expression in normal, HARM premalignant, and malignant oral cells following exposure to CS or administration of BRB phytochemicals. Using SABiosciences Human Cancer PathwayFinder PCR Array (cat. no. PAHS-033Z), 84 genes belonging to six biological pathways of transformation and tumorigenesis were evaluated for differential changes in expression between BRB-E and CS-E treated samples within each cell line. The purpose of this exploratory study was to learn more about the opposing roles CS and BRBs play in oral carcinogenesis, and search for possible mechanisms by which BRBs may counteract harmful changes in gene expression induced by CS.

RT-QPCR METHODS

Eight Human Cancer PathwayFinder PCR Array plates were used to evaluate the expression of 84 cancer-associated genes in 30 samples. Each 384-well plate tested 96 genes (84 cancer-associated, 12 housekeeping or positive control, **Table xv,** Appendix) in four different samples. See **Figure 8** for array plate schema. Because only 30 samples could be tested, biological duplicates were pooled into single samples, only one control sample per cell line from BRB-E experiments was used, and samples treated with the highest CS-E dose (20%) were not evaluated due to toxicity concerns. **Table ii** (Appendix) displays the composition of each sample tested in the array.

ANALYSIS OF GENE ARRAY DATA

Qiagen Data Analysis Center was used to obtain fold-changes in gene expression between each dose and a control. Raw data can be found in **Tables iii-vii,** Appendix. Before analysis, the samples treated with the highest doses (800µg/mL BRB-E or 10% CS-E) included in the array were removed due to unreliably high fold-changes resulting from possible cell death due to toxicity. From the samples that were analyzed, fold-changes for every gene compared to the control group can be found in **Tables ix-xiii.**

Ingenuity Pathway Analysis (IPA) software was used to identify possible relationships, mechanisms, functions and pathways present within the dataset. IPA uses the Ingenuity Knowledge Base, which unites findings published in more than 4,000 journals to make a database with comprehensive, up-to-date information on biological interactions from millions of individually studied relationships between proteins, genes, cells, drugs, diseases, and more. Data on 2-fold or greater changes in gene expression resulting from either 400 µg/mL BRB-E or 5% CS-E treatment were uploaded into IPA. This BRB-E dose was chosen based on previous studies (data not shown), and the 5% CS-E dose was chosen for analysis because it induced the most pronounced changes in gene expression without overt evidence of cytotoxicity. The Core Analysis function was used to classify the genes into possible biological pathways, networks, and mechanisms. This study elaborates on selected points of interest emerging from the IPA.

RESULTS AND CONCLUSIONS:

INTERPRETING INTERACTION NETWORKS

Solid lines indicate validated interactions, and dotted lines indicate predicted interactions. Increased expression is represented by the color red, and decreased expression is represented by green.

Normal Oral Cells (TE-1177)

BRB-E Treatment

BRB-E treatment did not induce any 2-fold or greater changes expression in any gene tested (0/84).

CS-E Exposure

IPA categorized the genes modulated into five networks; the majority belonged to one of three networks **(Table 7).**

- **Network 1:** Cell Death and Survival, Organismal Injury and Abnormalities, Cardiovascular System Development and Function
- **Network 2:** Developmental Disorder, Neurological Disease, Organismal Survival
- **Network 3:** Cancer, Hematological Disease, Organismal Injury and Abnormalities.

Of the genes modulated, 15 belonged to Network 1. **Table 7** lists all molecules and genes in the network, and the genes tested are in bold. With a few exceptions, most of the genes are upregulated following CS-E exposure. **Figure 9** displays an interaction network of differentially expressed genes. The gene *HMOX1* (heme oxygenase 1), which exhibited a large decrease in expression (21-fold down regulation), appears central to the network. According to the Ingenuity Knowledge Base (IKB), the gene plays a role in cellular response to hypoxia. Hypoxia, or oxygen deficiency, is a known consequence of CS exposure. A 5% dose of CS-E drastically reduced its expression, interfering with the cells' intake of oxygen. Likewise, modulation of *ANGPT2* (angiopoietin 2) is functions in important aspects of metastasis – cell migration, proliferation, and adhesion. It is a known feature in many cancers. It exhibits a striking 17-fold increase in expression in the CS-E treated normal cells.

Direct interactions in Network 2 are displayed in **Figure 10.** The direction of the modulations induced by CS-E in this network are more mixed. Some transcriptional repressors tested in this experiment (*SNAI1, SNAI2)* are predicted to be key elements in this network. Another transcriptional regulator, *SOX10,* showed a huge increase in expression – more than 38-fold. *SOX10* functions to regulate chromatin- and DNA-binding; it is important to cell differentiation, survival, and progression through the cell cycle (IKB). Its extreme upregulation may be indicative of a disturbance in the regulatory mechanisms for cell growth and division.

Network 3 is not displayed because genes tested in this experiment comprise only a small fraction of the network. In addition, the changes in expression of the bolded genes do not approach the magnitude of those highlighted in the previous two networks. However, both *AURKA* (aurora kinase A) and *CCL2* (C-C motif

chemokine ligand 2) have several validated and predicted interactions within the network. The protein encoded by *CCL2* recruits immune cells to sites of tissue damage. Its downregulation may mean that CS-E is working to inhibit the epithelium's ability to respond to and repair damage. *AURKA* functions in apoptosis, cell division, and cell cycle progression, and modulations of the gene have been associated with various cancers, including epithelial (IKB).

The genes that are modulated in these three networks within normal epithelial cells are involved in cell division and proliferation, as well as tissue damage repair. The disturbance of normal expression patterns in these processes by CS-E could very well translate into the initiation of multistep carcinogenesis.

HARM Premalignant Oral Cells (SCC-83)

While BRB-E did not have any significant effect on gene expression in normal cells, it induced interesting changes in HARM premalignant cells due to their abnormal, pre-cancerous state. Because they represent a phase in the multistep progression that is in between normal and premalignant, they appeared to initiate a mixture of normal and malignant-like responses to CS-E damage. The HARM cells were also susceptible to more severe fold-changes in response to CS-E. Because there were so many interesting responses in these cells, this analysis is focused only on expression changes that occurred in response to both treatments in opposite directions. It is notable that not a single gene was upregulated by BRB-E; all changes were downregulations. There were 30 genes that were both downregulated by BRB-E and upregulated by CS-E (See **Table 8**). IPA categorized genes modulated by BRB-E primarily into three networks (**Table 9**):

- **Network 4:** Cardiovascular System Development and Function, Cellular Movement, Connective Tissue Development and Function
- **Network 5:** Cell Death and Survival, Gastrointestinal Disease, Hepatic System Disease
- **Network 6:** Cardiovascular Disease, Cardiovascular System Development and Function, Embryonic Development.
-

The majority of genes modulated by CS-E belonged to four networks (**Table 10**):

- **Network 7:** Cell Death and Survival, Cell Morphology, Cellular Function and Maintenance.
- **Network 8:** Cardiovascular System Development and Function, Organismal Development, Tissue Morphology
- **Network 9:** Cell Death and Survival, Cellular Development, Cellular Growth and Proliferation.
- **Network 10:** Cell Cycle, Cell Morphology, Cellular Assembly and Organization

Within Networks 4 and 8 (**Figures 11, 12**) there were exciting differential changes in gene expression. *ANGPT1*, *CCL2, KDR, IGFBP3,* and *IGFBP5* were among some of the genes regulated in opposite directions by the two treatments. *CCL2,* which encodes a cytokine, features prominently in the Network 4 (**Figure 11**); it has the second greatest number of interactions. The cytokine is involved in functions important to abnormal cell growth in the body, like cell migration, recruitment, attraction, activation, etc. It has been studied in the context of many cancers and is implicated in tumorigenesis, so it is notable that its expression is both decreased by BRB-E and increased by CS-E. Also important is the gene *KDR* (kinase insert domain receptor),

which interacts with a multitude of molecules in Network 8 (**Figure 11**). It functions in important carcinogenesis-related processes (migration, proliferation, apoptosis, cell growth, cell death, etc.) and has been studied in the context of hundreds different tumors and abnormal tissue growths (IKB). In this experiment, the gene was hardly expressed by cells not treated with CS-E. Interestingly, BRB-E treatment prompted a 5-fold decrease in expression, while CS-E induced a greater than 1,000-fold increase in *KDR* expression. *IGFB3* and *IGFB5* (insulin-like growth factor-binding proteins 3 and 5) form numerous interactions in both networks, and have been associated with many cancers, including epithelial cancer (IKB). They are implicated in processes including apoptosis, cell proliferation, growth, survival, and migration, (IKB) which are functions that greatly impact abnormal cell growth and carcinogenesis.

More intriguing overlap occurred between Networks 5 and 7 (**Figure 13**, **Figure 14**), which encompass processes of cell death and survival in BRB-E and CS-E treated HARM cells. *CASP9* regulates the protein caspase-9 which initiates apoptosis and inflammation (IKB), and it is central in both networks. The differential change in expression is conspicuous; it is decreased 2-fold by BRB-E, and it is increased 12-fold by CS-E. This major upregulation is indicative of a defensive response – the HARM cells are signaling apoptosis in response to damage by CS-E. *EPO* (erythropoietin) is another gene that is downregulated by BRB-E and drastically upregulated by CS-E. Its primary function in the human body is to stimulate red blood cell production, and it has also been reported to stimulate angiogenesis (formation of new blood vessels). It is important to cell proliferation and survival (IKB). In this experiment, CS-E yielded a nearly 2,500-fold increase in *EPO* expression. This is especially alarming, because it means that CS-E treatment at this dose promoted the growth and proliferation of abnormal HARM cells – such activity in the human body conceivably leads to uncontrolled cell growth and the development of cancer. *CCND2* (CDK4 Cyclin-D2) is also an interesting feature of these two networks. It is clearly central to Network 5, and its bright color indicates that it underwent a relatively dramatic decrease in expression in response to BRB-E. It also forms many interactions in Network 7 and is upregulated 80-fold by CS-E. Like many other genes discussed, it is important in cell proliferation and cell cycle progression, and has been studied in the context of many cancers and diseases (IKB).

Malignant Oral Cells (CA-83)

Expression changes in the previous two cell lines mostly fell into consistent patterns: BRB-E decreased expression, and CS-E increased expression. Like HARM premalignant cells, malignant cells were very responsive to treatment by both BRB-E and CS-E. However, in these tumorigenic cells, changes caused by either treatment were slightly less predictable in their directionality. In addition, there appeared to be fewer genes responsive to both treatments, and many more that drastically changed in expression in response to only one of the treatments. See **Table 11** for genes that exhibited 2-fold or greater changes in expression.

The genes regulated by BRB-E fit mostly into two networks (**Table 12**):

- **Network 11:** Cell Death and Survival, Cell Cycle, Cancer
- **Network 12:** Embryonic Development, Cell Death and Survival, Organismal Injury and Abnormalities.

Genes regulated by CS-E were also primarily categorized into two networks (**Table 13**):

- **Network 13:** Cardiovascular System Development and Function, Cellular Movement, Cell Morphology.
- **Network 14:** Cell Death and Survival, Organismal Injury and Abnormalities, Gastrointestinal Disease.

Consistent with the pattern, CS-E upregulated nearly all of the genes tested. But BRB-E, which produced decreases in expression in almost all of the cancer-associated genes in normal and HARM cells, caused irregular changes in malignant cells. Some genes that were upregulated by BRB-E exhibited even greater increases with CS-E (*CA9, CCL2, CCND2, FASLG*). Others appreciably increased in expression in response to BRB-E, but did not even break the two-fold threshold when treated with CS-E.

CHAPTER FIVE: DISCUSSION

The goal of this study was to explore the effects of BRB-E and CS-E on normal, HARM premalignant, and malignant oral cells. Additionally, these experiments were conducted with the intention of identifying potential biomarkers of both CS exposure and response to BRB intervention. BRBs present a possible mechanism of chemoprevention for the over one billion smokers that are at risk for developing oral diseases, including cancer. Cancer chemoprevention can be thought of in terms of primary, secondary, and tertiary strategies. Primary chemoprevention aims to prevent the emergence of the disease in the first place. This can be through lifestyle, behavioral, and nutritional approaches that reduce exposures that increase the risk for oral disease and cancer. Never smoking and maintaining a lifelong commitment to a diet rich in fruits, such as black raspberries, would exemplify primary chemoprevention. The normal oral cell line TE-1177 is a proxy for the average-risk "healthy" scenario. Secondary chemoprevention focuses on mitigating the impact of oral disease/cancer once it is already present. People who are former smokers (or possibly current smokers) who are on a purposeful diet enriched for BRBs, or maybe a yet to be identified bioactive components of BRBs would be a model for this phase of oral cancer prevention. The HARM oral cells represent this category of enhanced risk oral tissues that are not quite malignant, but not longer normal. Tertiary cancer chemoprevention attempts to attenuate to impact of the disease in progress as well as in the specific case of oral cancer, reduce the risk of future oral cancer (recurrence). This type of chemoprevention overlaps in practice with chemotherapeutic strategies, since treatment of the disease is the primary endpoint. The malignant oral cell line CA-83 is used as a surrogate for this state. Previous studies19,20 have defined four pillars of an effective chemoprevention intervention. I will discuss my study in the context of these four pillars:

- **First**, identify *high at-risk populations* whose cancer risk warrants intervention.
- **Second**, identify *effective agents* that can reasonably be administered over a long period of time in people who have not yet developed cancer.
- **Third**, *discover and validate biomarkers* that can predict the response to intervention.
- **Fourth**, develop a cost-conscious product that can easily be distributed to target populations.

HIGH AT-RISK POPULATION

BRB chemoprevention targets the population at highest risk for the development of oral cancer due to tobacco smoke exposure. Because smoking exposes the entire oral cavity to the carcinogens present in a burning cigarette, a broad "field of cancerization" is created with many *potentially* malignant oral cells. Many of these cells show only molecular damage without an observable change in morphology or phenotype. These HARM cells represent a premalignant state that have lost some aspect of normal cell growth control, but still maintain enough biochemical resolve to not commit to malignant progression. As stated previously, this at-risk group constitutes about 15% of the U.S. population, and over 1 billion people globally. The disease disproportionally impacts disadvantaged populations, which applies broadly to developing countries experiencing rising rates of smoking and lacking public health infrastructure. However, it is also applicable to populations within the U.S. more likely to smoke due to socioeconomic or educational status. Also, African American males are far more likely to die from oral cancer than White males, despite similar disease incidence

and current smoking rates: Data from 1993–2003 show that during this time period, the 5-year survival rate for white males was 61.2% versus only 35% for black males.13 An effective *prevention* strategy must address the populations that are especially susceptible to oral cancer incidence and death, including tackling disparities in SES, access to healthcare (especially screening and disease surveillance), and education in risk reduction. An efficient *chemopreventive* strategy must complement such a framework and provide an applicable and effective intervention to those highest at risk for oral cancer development.

EFFECTIVE AGENT

As previously discussed, BRBs have consistently supported the definition of a "functional food" for health. Functional foods provide a health advantage in excess of their conventional nutritional complement of vitamins and minerals. BRBs are a rich source of vitamins, minerals, micronutrients and fiber, and yet this simple list does not explicitly account for their ability to reduce oral cancer incidence in preclinical studies. Somewhere in the complex phytochemical cocktail present within BRBs is a combination of compounds that inhibit aerodigestive tract cancers. BRBs administered as a dietary *chemopreventive* have repeatedly demonstrated their ability to prevent oral and other cancers of the aerodigestive tract in experimental models. Importantly, as opposed to a *chemotherapeutic*, BRB studies have repeatedly shown that there is no evidence of toxicity associated with administration of BRBs.21-23, 25-28.

BIOMARKERS CAN INDICATE RISK AND PREDICT RESPONSE TO INTERVENTION.

Biomarkers have the potential to be prognostic tools for evaluating one's risk for oral cancer well in advance of the overt appearance of the disease. They can also function as predictors of one's response to an intervention, like BRB's. These *in vitro* studies validated the clinical biomarker *TRNP1* as an *in vitro* biomarker of CS-exposure in HARM oral cells, and also introduced many other cancer-associated genes that could play a prognostic or predictive role. Of the genes discussed, those that drastically changed in expression in response to CS-E in normal cells (*HMOX1, ANGPT2, AURKA, CCL2, FASLG*, etc.) could serve as biomarkers of CS exposure in otherwise healthy tissue. This could be a tool used by dentists and clinicians to reliably evaluate a patient's smoking status.

In both HARM premalignant and malignant cells, the two treatments elicited many notable changes in gene expression. The genes that exhibited changes induced by the two treatments in opposite directions are candidates for further studies evaluating the interaction effects of CS exposure and BRB-E treatment. In addition, these biomarkers provide insight into the pathways and possible mechanisms of oral carcinogenesis, as well as possible mechanisms of action taken by BRB treatment that could inhibit the multi-step process of carcinogenesis.

PRODUCT

BRBs as a whole food have already been incorporated in products like lozenges, extracts, powders, confections, and drinks (nectars). BerriHealth is a small company with the mission of supplying customers with "quality black raspberry products to help improve their quality of life." Their products can be consumed alone,

or mixed with other foods like smoothies, cereal, or yoghurt. Translational scientist and clinicians at The Ohio State University Comprehensive Cancer Center – The James (OSUCCC–The James) have developed a BRB confection (Food Fight: Morsels That Take A Bite Out Of Cancer)(Gu J, Ahn-Jarvis JH, Vodovotz Y. Development and characterization of different black raspberry confection matrices designed for delivery of phytochemicals. J Food Sci 80(3):E610–618. 2015) for use in combating oral cancer. Unfortunately, BRB products are somewhat expensive, and BRBs themselves are not easily accessible in certain geographic areas. For this reason, current research continues to explore the effectiveness of other berry fruits that are cheaper and more widely available. Consequently, researchers at OSUCCC–The James have also developed a novel ripe whole strawberry-based confection (New Research Investigates Strawberries to Fight Oral Cancer in Heavy Smokers) for use in combating oral cancer risk in otherwise healthy smokers. Importantly, food-based interventions do not have to be an “either-or” decision, and it is hypothesized that the unique combination of phytochemicals present in BRBs and strawberries can work synergistically to decrease cancer risk. It is also worth considering that if the efficacy of BRBs continues to be confirmed, then the demand for BRBs will increase, and methods for widespread production could be implemented to make them inexpensive and available around the world.

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TABLES

Table 1. Estimated new cases and deaths for oral cavity and pharynx cancer in 2016.[1]

Total (new cases)	**Male**	**Female**
48,330	34,780	13,550
Total (deaths)	**Male**	**Female**
9,570	6,910	2,660

Table 2. Comparison of TE-1177, SCC-83, and CA-83 epithelial cell lines

Normal Phenotype (TE-1177)	**HARM Premalignant Phenotype (SCC-83)**	**Malignant Phenotype (CA-83)**
Finite lifespan with senescence	Infinite lifespan (immortalized), transformed	Infinite lifespan (immortalized), transformed
Anchorage-dependent	Anchorage-independent	Anchorage-independent
Does not grow tumors in nude mice	Does grow tumors in nude mice	Grows tumors in nude mice

Table 3: Literature Review: *In Vitro* Cigarette Smoke Experiment

Source	Vacuum		Extract Stability		Filter Sterilization		Cigarette Filter		Cigarettes Type			Extract Concentration			Vehicle		
Author, Year	Yes	No	Immediate	Archive	YES	NO	YES	NO	Reference	Retail	<1 cigarette/5mL	>1 cigarette/5mL	% Concentrations	Serum Medium	Serum-free Medium	Saline
Le Rouzic, 2016		•1	•		•		•		•		2 cigarettes/10 mL		4	•		
Nie, 2016	•2		•		•		•			•		5 cigarettes/10 mL				
Heulens, 2016			•		•		•		•		1 cigarette/10 mL		10, 25			
Liu, 2016		•3	•		•			•		•		3 cigarettes/3 mL	0.5, 1, 2, 5 diluted with	•		•
Zhang, 2016		•1	•		•			•	•		3 cigarettes/25 mL		0, 1, 5, 10, 15, 20			
Siebos, 2007	•4		•			•		•			4 cigarettes/20 mL					
Li, 2016	•			•		•	•			•		10 cigarettes/10 mL	0.125, 0.25, 0.5, 1, 2, 4,			
Lacy, 2016			•		•		•		•		2 cigarettes/20 mL		0, 16.25, 32.5 OD		•	
Poussin, 2014		•5	•			•	•		•		10 cigarettes/32 mL; 2.5 puffs/mL		0.015-0.18 puffs/mL			
Hoshino, 2001			•			•	•					5 cigarettes/10 mL				
Wirtz, 1996	•		•1		•		•		•		4 cigarettes /100 mL					
11	4	4	10	1	7	4	8	3	7	3						

Source	Adjusted pH		Absorbance		Flow Rate		Vehicle		
Author, Year	YES	NO	YES	NO	YES	NO	Serum Medium	Serum-free Medium	Saline
Le Rouzic, 2016		•		•		•	•		
Nie, 2016	•			•	•1				
Heulens, 2016	•		•1			•	•		
Liu, 2016	•		•2			•			•
Zhang, 2016	•			•		•	•		
Siebos, 2007	•			•		•	•		
Li, 2016	•		•3			•		•	
Lacy, 2016	•		•3			•			
Poussin, 2014		•		•		•			•
Hoshino, 2001				•	•1		•		
Wirtz, 1996			•3		•2		•		
	8	2	5	6	3	8	7	2	2

1 - 340nm
2 - 270, 280nm
3 - 320nm

1 - 0.3L/min
2 - 3min/cigarette

Unknown

1 - Syringe
2 - Compressor
3 - Gas Collection Pipe
4 - Peristaltic Pump
5 - Smoking Machine

1 - Incubated at 37°C, 90 min

Table 4: Clinical biomarker signature assessed in this study.

Gene	Protein Encoded
ACTB	β-actin, a cytoskeletal protein involved in cell structure and motility. It is commonly used as a reference gene.
DUSP1	Dual specificity protein phosphatase 1, an enzyme produced human skin fibroblasts in response to oxidative or heat stress and growth factors.
KRT10	Keratin 10, a cytoskeleton protein.
ALOX12B	Arachidonate 12-lipoxygenase, 12R type, an enzyme that may participate in fatty acid metabolism in the skin epidermis.
HTR3A	5-hydroxytryptamine receptor 3A is a subunit of serotonin, which functions as a neurotransmitter and hormone.
CD207	Expressed in only in Langherhan's cells (antigen-presenting dendritic cells), which are found in increased numbers in the oral mucosa of smokers.
TRNP1	TMF-regulated nuclear protein 1, which regulates cell growth and chromatin remodeling
PNLRP1	Pancreatic lipase related protein 1

Table 5. Changes in gene expression following CS exposure (± SD).

Fold Change (2^-ΔΔCT)

	ACTB	TRNP1
TEE CSE1.25	1.28	1.47
TEE CSE2.5	2.44	1.75
TEE CSE5	0.40	1.12
TEE CSE10	0.55	0.29
TEE CSE20	0.23	0.06
Global TEE	0.83	0.77

Fold Change (2^-ΔΔCT)

	ACTB	TRNP1
SCCCSE1.25	0.09	0.47
SCCCSE2.5	1.89	10.24
SCCCSE5	2.14	17.14
SCCCSE10	1.22	3.40
SCCCSE20	1.31	8.71
Global SCC	0.92*	8.38*

*p<0.01

Table 6. Changes in gene expression following BRB-E treatment

Fold Change (2^-ΔΔCT)

		ACTB	TRNP1
LOW	**TEE BRB 2**	5.9E-05	0.71
	TEE BRB 3	0.28	0.73
	Global TEE Low	0.07	0.72
HIGH	**TEE BRB 5**	0.001	0.04
	TEE BRB 6	0.05	0.25
	Global TEE High	0.01	0.13

Fold Change (2^ΔΔCT)

		ACTB	TRNP1
LOW	**SCC BRB 2**	0.52	0.10
	SCC BRB 3	0.37	0.12
	Global SCC Low	0.44	0.0004
HIGH	**SCC BRB 5**	0.08	0.11
	SCC BRB 6	0.30	0.23
	Global SCC High	0.17	0.16

Table 7. Primary Networks in CS-E treated TE-1177.

Analysis	Molecules in Network	Score	Focus Molecu	Top Diseases and Functions
5 CSE	ADRB, AMPK, ↓**ANGPT1,** ↑**ANGPT2,** Ap1, ↑**BCL2L11,** Cbp/p300, Collagen Alpha1, cytochrome C, ↑**DDIT3,** ↑**EPO,** ERK1/2, ↑**ETS2,** ↑**FASLG,** Fibrin, Fibrinogen, ↓**G6PD,** GOT, ↑**GSC,** ↓**HMOX1,** ↑**IGFBP3,** ↑**MAP2K3,** N-cor, NADPH oxidase, NFAT (complex), p70 S6k, PARP, PDGF BB, ↑**PGF,** PI3K (family), ↑**POLB,** SAA, Smad2/3-Smad4, ↑**TEK,** TSH	35	15	Cell Death and Survival, Organismal Injury and Abnormalities, Cardiovascular System Development and Function
5 CSE	↓**ADM,** Akt, Alp, Alpha catenin, ↑**CA9,** calpain, ↑**CDH2,** Collagen type I, Collagen(s), Cyclin A, ↑**GADD45G,** ↓**GPD2,** Growth hormone, hemoglobin, IgG, Igm, Immunoglobulin, ↓**KDR,** Laminin, Ldh (complex), LDL, N-Cadherin, Pdgf (complex), PI3K (complex), PLC gamma, ↑**PPP1R15A,** ↑**SNAI1,** ↓**SNAI2,** Sod, ↑**SOX10,** STAT5a/b, Tgf beta, ↓**TNKS,** ↓**TNKS2,** Wnt	26	12	Developmental Disorder, Neurological Disease, Organismal Survival
5 CSE	ADCY, ↑**AURKA,** ↑**BIRC3,** ↓**BMI1,** CaMKII, caspase, ↓**CCL2,** CD3, Cg, Ck2, Creb, ↑**DDB2,** Focal adhesion kinase, Hdac, Histone h3, Histone h4, Hsp70, IL1, IL12 (complex), ↑**KRT14,** MAP2K1/2, Mek, Nfat (family), NFkB (complex), Nos, ↑**PINX1,** Pkc(s), Raf, Ras, Ras homolog, RNA polymerase II, Sos, ↑**STMN1,** TCR, Tnf (family)	16	8	Cancer, Hematological Disease, Organismal Injury and Abnormalities
5 CSE	26s Proteasome, ↓**ACSL4,** Arnt-Hif1a, ↑**CDC20,** chemokine, CHRM3, ↑**DSP,** ELK3, ERK, Fetal Hemoglobin, FSH, GAS6, GCLC, Gsk3, IL12 (family), Insulin, Interferon alpha, Jnk, KCNB1, ↓**LPL,** Mapk, MIDN, Mmp, N,N-dimethylarginine, NCFR, P38 MAPK, p85 (pik3r), Pka, Pro-inflammatory Cytokine, Proinsulin, Rac, Shc, SRC (family), Vegf, VTCN1	7	4	Cell-To-Cell Signaling and Interaction, Cellular Assembly and Organization, Cellular Function and Maintenance
5 CSE	↓**LIG4,** RUVBL2, ↓**SNAI3,** STRIP1, STRN3	5	2	Cell Morphology, Cellular Assembly and Organization, DNA Replication, Recombination, and Repair

Table 8. Genes modulated in opposite directions by BRB-E and CS-E in HARM premalignant cells.

	SCC-83 400 BRB Fold Change Compared to Control Group	SCC-83 5% CS-E Fold Change Compared to Control Group
ACLY	-2.486325211	50.9537
ANGPT1	-4.30292599	36.7527
ANGPT2	-2.715177844	122.8402
ARNT	-2.809778028	14.8521
AURKA	-1.343363783	2.9948
BIRC3	-2.160760588	7.5851
CA9	-3.657644477	309.0038
CASP9	-2.411963338	11.9847
CCL2	-2.719608376	502.8627
CCND2	-4.970178926	81.0403
CPT2	-2.181976871	8.8557
DDIT3	-2.15285253	27.4693
EPO	-4.55996352	2471.756
FASLG	-5.367686527	858.2455
FLT1	-4.395604396	533.7434
GADD45G	-6.165228113	583.0839
GSC	-3.438789546	837.3034
IGFBP3	-3.988831272	33.062
IGFBP5	-4.930966469	19.453
KDR	-5.249343832	1173.5777
KRT14	-4.985044865	1799.7286
LPL	-4.906771344	1358.1213
OCLN	-3.70096225	52.386
SERPINB2	-2.009242516	37.7811
SERPINF1	-3.059039462	30.2877
SNAI1	-4.486316734	400.7875
SNAI3	-3.487966516	48.7376
SOX10	-3.206155819	507.3775
TEK	-7.225433526	682.4056
TERF1	-2.724053391	30.2482
HGDC	-5.06842372	2141.7608

Table 9. Primary Networks in BRB-E treated SCC-83.

Analysis	Molecules in Network	Score	Focus Molecu	Top Diseases and Functions
BRB_400	Alp, AMPK, ↓**ANGPT1,** ↓**ANGPT2,** calpain, ↓**CCL2,** Cyclin A, Cyclin D, cytochrome C, ERK1/2, ↓**FASLG,** Fibrin, Fibrinogen, GOT, ↓**GSC,** Ifn gamma, ↓**IGFBP3,** ↓**IGFBP5,** Laminin, NADPH oxidase, NFAT (complex), ↓**OCLN,** p70 S6k, PARP, PDGF BB, PI3K (family), Rb, ↓**SERPINF1,** Smad2/3-Smad4, ↓**SNAI1,** ↓**SOX10,** ↓**TEK,** Tgf beta, TSH, Wnt	28	12	Cardiovascular System Development and Function, Cellular Movement, Connective Tissue Development and Function
BRB_400	26s Proteasome, ↓**ACLY,** Akt, Ap1, ↓**ARNT,** BCR (complex), ↓**BIRC3,** ↓**CASP9,** caspase, Caspase 3/7, ↓**CCND2,** Cg, Ciap, ↓**DDIT3,** ↓**EPO,** ↓**GADD45G,** Hsp90, Igm, IL12 (complex), Immunoglobulin, Interferon alpha, ↓**KRT14,** LDL, Lh, ↓**MAP2K1,** MAP2K1/2, Mek, p85 (pik3r), PP1 protein complex group, PP2A, Shc, Sos, STAT5a/b, ↓**TBX2,** Ubiquitin	25	11	Cell Death and Survival, Gastrointestinal Disease, Hepatic System Disease
BRB_400	Alpha catenin, ↓**AURKA,** ↓**CA9,** CaMKII, Collagen type I, Collagen(s), ↓**CPT2,** Creb, estrogen receptor, ↓**FLT1,** Focal adhesion kinase, Growth hormone, Hdac, HDL, Histone h3, Histone h4, IL1, Insulin, Integrin, ↓**KDR,** ↓**LPL,** Mapk, Mmp, NFkB (complex), Nr1h, P38 MAPK, Pdgf (complex), PI3K (complex), PLC gamma, Ras, Ras homolog, RNA polymerase II, ↓**SERPINB2,** SRC (family), Vegf	14	7	Cardiovascular Disease, Cardiovascular System Development and Function, Embryonic Development
BRB_400	Adaptor protein 1, ARPP21, C1R, CCDC88B, CD3, CD52, CD70, Ck2, CLDN4, CMKLR1, ELK3, ERK, FSH, GBP1, Gsk3, HMGN2, IgG, IL12 (family), Jnk, MCL1, PCYT2, Pkc(s), Proinsulin, Rac, ↓**SNAI3,** STRN3, TCR, Tcrd, ↓**TERF1,** TNF, Tnf (family), trehalose, VIPR1, VIPR2, VTCN1	3	2	Cell Morphology, Hair and Skin Development and Function, Hematological System Development and Function

Table 10. Primary Networks in CS-E treated SCC-83.

Analysis	Molecules in Network	Score	Focus Molecu	Top Diseases and Functions
CSE_5	Akt, ↑**APAF1,** APC (complex), ↑**BCL2L11,** BCR (complex), ↑**CASP2,** ↑**CASP7,** ↑**CASP9,** caspase, Caspase 3/7, Caspase 3/6/7, ↑**CCND2,** ↑**CCND3,** Cdk, ↑**CFLAR,** Ciap, Cyclin A, cytochrome C, death receptor, DNAJ, ↑**DSP,** EGLN, ↑**EPO,** ↑**FASLG,** Gm-csf, HLA-DR, Ifn gamma, Igm, Mek, N-Cadherin, ↑**NOL3,** PARP, Rb, ↑**SKP2,** ↑**WEE1**	28	14	Cell Death and Survival, Cell Morphology, Cellular Function and Maintenance
CSE_5	Adaptor protein 1, ↑**ANGPT1,** Collagen Alpha1, creatine kinase, Cyclin D, Cyclin E, ERK1/2, ETS, ↑**ETS2,** Ferritin, Fgf, ↑**FGF2,** Fibrin, Fibrinogen, ↑**FLT1,** ↑**FOXC2,** Growth hormone, ↑**GSC,** Igfbp, ↑**IGFBP3,** ↑**IGFBP5,** ↑**KDR,** NADPH oxidase, Neuropilin, PDGF-DD, ↑**PGF,** PLC gamma, SAA, Scf, ↑**SERPINF1,** Smad2/3, Smad2/3-Smad4, ↑**TEK,** TSH, Wnt	23	12	Cardiovascular System Development and Function, Organismal Development, Tissue Morphology
CSE_5	14-3-3, ↑**ADM,** ↑**ANGPT2,** ↑**BIRC3,** ↑**CA9,** Calcineurin protein(s), CaMKII, Cdc2, Creb, ↑**DDB2,** Fcer1, ↓**G6PD,** ↑**GADD45G,** glutathione peroxidase, Hsp27, Ldh (complex), ↑**MAP2K1,** ↑**MAP2K3,** MAP2K1/2, MAP3K, ↑**MAPK14,** NFAT (complex), Nfat (family), NFkB (complex), NFkB (family), Notch, Pak, PI3K (family), Ptk, Raf, Rxr, Sapk, ↑**SERPINB2,** Sod, TCR	21	11	Cell Death and Survival, Cellular Development, Cellular Growth and Proliferation
CSE_5	↑**ACLY,** Alpha catenin, Ap1, ↑**ARNT,** ↑**AURKA,** calpain, ↑**CCL2,** Collagen type I, estrogen receptor, Focal adhesion kinase, Gsk3, Integrin, Jnk, Laminin, Mapk, ↑**MKI67,** Mmp, NMDA Receptor, ↑**OCLN,** p85 (pik3r), Pdgf (complex), PI3K (complex), ↑**PINX1,** Pkc(s), PP1 protein complex group, Pro-inflammatory Cytokine, Rac, Ras, Ras homolog, ↑**SNAI1,** Sos, ↑**TERF1,** ↑**TINF2,** Tnf (family), ↑**TNKS**	21	11	Cell Cycle, Cell Morphology, Cellular Assembly and Organization
CSE_5	ADCY, ADRB, Alp, AMPK, Cbp/p300, Collagen(s), ↑**CPT2,** ↑**DDIT3,** ERK, ↑**GPD2,** HDL, hemoglobin, HISTONE, ↑**HMOX1,** Ige, IgG, Ikb, IL1, LDL, ↑**LPL,** N-cor, Nos, Nr1h, p70 S6k, PDGF BB, PEPCK, PP2A, ↑**PPP1R15A,** Proinsulin, Rar, ↑**SNAI2,** ↑**SOX10,** STAT5a/b, Tgf beta, Ubiquitin	14	8	Cellular Development, Gastrointestinal Disease, Hepatic System Disease
CSE_5	26s Proteasome, Actin, Calmodulin, CD3, Cg, chemokine, Ck2, ↑**DKC1,** ↑**ERCC3,** ↑**ERCC5,** FSH, GOT, Hdac, Histone h3, Histone h4, Hsp70, Hsp90, IKK (complex), IL12 (complex), IL12 (family), Immunoglobulin, Insulin, Interferon alpha, ↑**KRT14,** Lh, P38 MAPK, ↓**PFKL,** Pka, PLC, ↓**POLB,** RNA polymerase II, Shc, ↑**SOD1,** SRC (family), Vegf	12	7	Cancer, Dermatological Diseases and Conditions, Developmental Disorder

Table 11: Two-fold or greater changes in BRB-E or CS-E treated CA-83

	SCC-83 400 BRB	SCC-83 5% CS-E
	Fold Change Compared to Control Group	**Fold Change Compared to Control Group**
ACLY		28.1292
ACSL4	-55.55555556	-2.783964365
ADM	-15.64945227	4.2192
ANGPT1		38.0224
ANGPT2	2.079	5.7455
APAF1	-8.741258741	
ARNT	-2.186270223	
ATP5A1	-64.51612903	
AURKA	-17.73049645	
BCL2L11	-8.006405124	4.947
BIRC3	-4.926108374	
BMI1	-19.37984496	
CA9	2.3903	302.9215
CASP2	-22.32142857	
CASP7	-21.32196162	
CASP9	-2.808988764	
CCL2	5.4386	208.0794
CCND2	3.6565	86.374
CCND3	11.8335	
CDC20	2.5153	
CDH2	2.4379	
CFLAR	-36.63003663	
COX5A	-178.5714286	
CPT2	3.2087	
DDIT3		
DKC1	5.3267	8.1318
EPO		115.0162
ERCC5	-2.843332386	115.0162
ETS2	-4.636068614	
FASLG	23.5558	77.5504
FGF2	-7.153075823	
FLT1	-87.71929825	1074.7847
FOXC2		3.0066
G6PD		-2.435460302
GADD45G		351.9742
GSC		337.1178
IGFBP3	25.3172	7.2688
IGFBP5	-8.23723229	

IGFBP7	2.1044	
KDR		722.3975
KRT14	2948.0156	102.2128
LPL		449.7739
MAP2K3		2.9764
NOL3	19.2644	
OCLN	-149.2537313	
PGF	-909.0909091	
PINX1	75.7727	2.2771
PPP1R15A		5.4411
SERPINB2		11.383
SERPINF1		4.5117
SNAI1	-2.275312856	
SNAI2	-370.3703704	8.6504
SNAI3	131.4596	
SOX10	914.5318	
STMN1	4.1441	
TERF1		8.6504
TNKS2	245.8592	
UQCRFS1	-18.76172608	
VEGFC	-3.39673913	
WEE1	2.8597	

Table 12. Primary Networks in BRB-E treated CA-83.

Analysis	Molecules in Network	Score	Focus Molecu	Top Diseases and Functions
BRB 400	Adaptor protein 1, ↓**APAF1,** ↓**AURKA,** ↓**CASP2,** ↓**CASP7,** caspase, Caspase 3/7, Caspase 3/6/7, ↑**CCND2,** ↑**CCND3,** ↑**CDC20,** cdk, ↓**CFLAR,** ↓**COX5A,** Cyclin A, Cyclin D, Cyclin E, cytochrome C, death receptor, ERK1/2, ↓**FGF2,** Fibrin, Growth hormone, Igfbp, ↑**IGFBP3,** ↑**IGFBP7,** ↑**NOL3,** PARP, PDGF-DD, ↑**PINX1,** Rb, ↑**STMN1,** TSH, ↑**WEE1,** Wnt	37	16	Cell Death and Survival, Cell Cycle, Cancer
BRB 400	↓**ADM,** Akt, Alp, AMPK, ↑**ANGPT2,** ↓**ARNT,** ↓**ATP5A1,** ↓**BCL2L11,** BCR (complex), ↑**CA9,** ↓**CASP9,** Collagen type I, Collagen(s), EGLN, ↑**FASLG,** GOT, HDL, ↑**KRT14,** Laminin, LDL, MAP2K1/2, NADPH oxidase, NFAT (complex), Nos, Notch, p70 S6k, Pdgf (complex), PDGF BB, PI3K (family), ↑**PPP1R15A,** SAA, ↑**SERPINF1,** STAT5a/b, ↑**TNKS,** ↑**TNKS2**	29	13	Embryonic Development, Cell Death and Survival, Organismal Injury and Abnormalities
BRB 400	26s Proteasome, Alpha catenin, Ap1, ↓**BIRC3,** Calcineurin protein(s), CaMKII, ↑**CCL2,** CD3, ↑**DKC1,** ↓**ERCC3,** ↓**ERCC5,** Fgf, Fibrinogen, Gm-csf, HLA-DR, Hsp90, IgG, Igm, Ikb, IL1, IL12 (complex), IL12 (family), Immunoglobulin, Interferon alpha, Mek, Nfat (family), NFkB (complex), p85 (pik3r), PLC gamma, Shc, Sos, SRC (family), TCR, Tgf beta, Tnf (family)	9	5	Cancer, Dermatological Diseases and Conditions, Developmental Disorder
BRB 400	↓**ACSL4,** Actin, ADCY, Calmodulin, calpain, ↑**CDH2,** Cg, Ck2, ↑**CPT2,** Creb, ERK, estrogen receptor, ↓**FLT1,** Focal adhesion kinase, FSH, Gsk3, Hdac, Histone h3, Histone h4, Hsp70, Insulin, ↑**KDR,** Lh, Mapk, NMDA Receptor, Nr1h, P38 MAPK, PI3K (complex), Pka, Pkc(s), Proinsulin, Ras, Ras homolog, RNA polymerase II, Vegf	9	5	Cardiovascular System Development and Function, Embryonic Development, Organismal Development
BRB 400	ACKR3, AR, ATR, ↓**BMI1,** Cdkn1c, CNTF, CRH, DGCR8, DHH, DLX5, EDNRB, FOS, GAS6, GDNF, HHEX, ITGA6, Jnk, KRT8, KRT18, Map3k7, MED12, MED12L, Mmp, MMP13, NGFR, NMI, PAX3, PIK3R2, PKP2, POU3F3, ↑**SNAI3,** ↑**SOX10,** TCF12, TRADD, TSH	5	3	Embryonic Development, Organismal Development, Cell Death and Survival

Table 13. Primary Networks in CS-E treated CA-83.

Analysis	Molecules in Network	Score	Focus Molecu	Top Diseases and Functions
5 CSE	↑**ANGPT1,** Caspase 3/7, Cbp/p300, ↑**CCND2,** Ciap, CK1, Collagen Alpha1, Cyclin D, ↑**EPO,** ERK1/2, ↑**ETS2,** Fcer1, Fibrin, Fibrinogen, ↑**FLT1,** ↑**FOXC2,** ↑**GSC,** ↑**IGFBP3,** ↑**IGFBP5,** ↑**KDR,** Laminin, ↑**MAP2K3,** Mek, Neuropilin, NFAT (complex), PDGF BB, ↑**PGF,** PLC gamma, SAA, ↑**SERPINF1,** Smad2/3, Smad2/3-Smad4, Sos, TSH, Wnt	29	13	Cardiovascular System Development and Function, Cellular Movement, Cell Morphology
5 CSE	48s, ↑**ACLY,** ↑**ADM,** Akt, ↑**ANGPT2,** ↑**ARNT,** ↑**BCL2L11,** BCR (complex), ↑**CA9,** ↑**CFLAR,** Collagen(s), ↑**DDIT3,** ↑**FASLG,** ↓**G6PD,** GOT, Growth hormone, HDL, hemoglobin, ↑**HMOX1,** IgG, Igm, Ldh (complex), LDL, NADPH oxidase, Nfat (family), NFkB (family), Nos, PARP, PI3K (family), ↑**PPP1R15A,** Raf, Smad, Sod, Tgf beta, ↓**XIAP**	29	13	Cell Death and Survival, Organismal Injury and Abnormalities, Gastrointestinal Disease
5 CSE	Alp, AMPK, Ap1, ↓**BIRC3,** caspase, ↑**CCL2,** CD3, Cdk, chemokine, Cyclin A, cytochrome C, ↑**GADD45G,** Histone h4, Hsp70, Hsp90, Ifn gamma, IL1, IL12 (complex), IL12 (family), Immunoglobulin, Interferon alpha, Jnk, ↑**KRT14,** ↑**LPL,** MAP2K1/2, NFkB (complex), p70 S6k, PI3K (complex), Pro-inflammatory Cytokine, ↑**SERPINB2,** ↓**SKP2,** STAT5a/b, TCR, Tnf (family), Ubiquitin	13	7	Cellular Function and Maintenance, Organismal Survival, Cell Death and Survival
5 CSE	26s Proteasome, AHCY, ↓**ATP5A1,** ↑**CA9,** Ck2, CTSB, DDX3X, EEA1, EIF4B, FABP4, ↑**FOXC2,** HMGN2, HNRNPD, MED31, Mg2+, Nedd4, NME2, ↑**PINX1,** PTGDS, PTPN6, RBBP4, RNA polymerase II, RPL18A, RRAD, SAT1, SKIL, SMARCC2, SP3, SRPK1, ↑**TERF1,** THRA, Top2, TP53, TXNIP, UTP14A	9	5	Cancer, Connective Tissue Disorders, Organismal Injury and Abnormalities
5 CSE	↓**ACSL4,** ADRB, Calmodulin, calpain, Cg, Collagen type I, ↑**CPT2,** Creb, ↓**DSP,** ERK, estrogen receptor, Focal adhesion kinase, FSH, Gsk3, Hdac, Histone h3, Insulin, Lh, Mapk, ↓**MKI67,** Mmp, Nr1h, P38 MAPK, p85 (pik3r), Pdgf (complex), Pka, Pkc(s), PP1 protein complex group, Proinsulin, Rac, Ras, Ras homolog, Shc, SRC (family), Vegf	7	4	Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry

FIGURES

Figure 1. Full experimental plate schema.

Figure 2. University of Kentucky 1R6F Reference Cigarettes.

Figure 3. Stylistic representation of custom smoking apparatus.

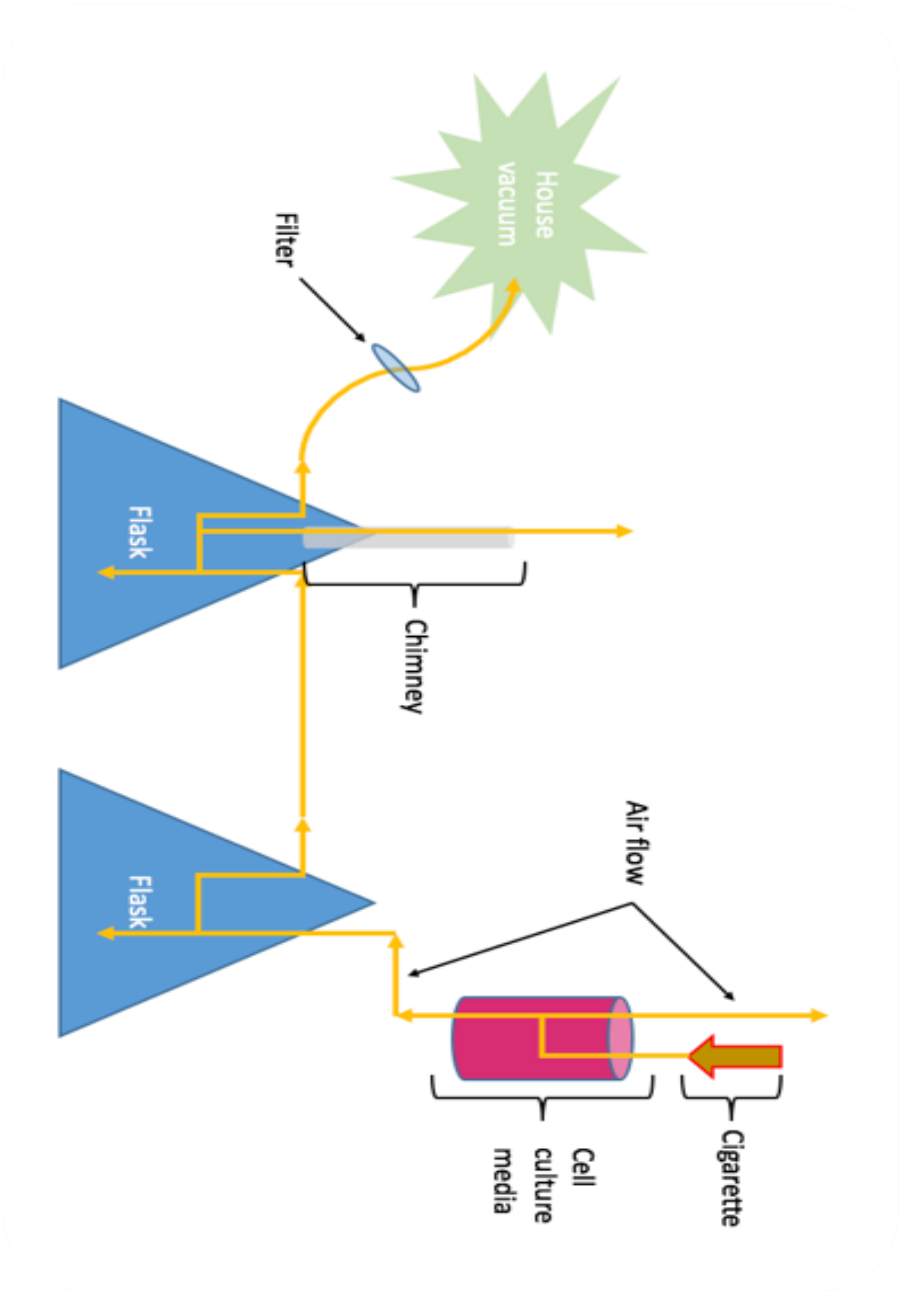


Figure 4. Custom smoking apparatus.

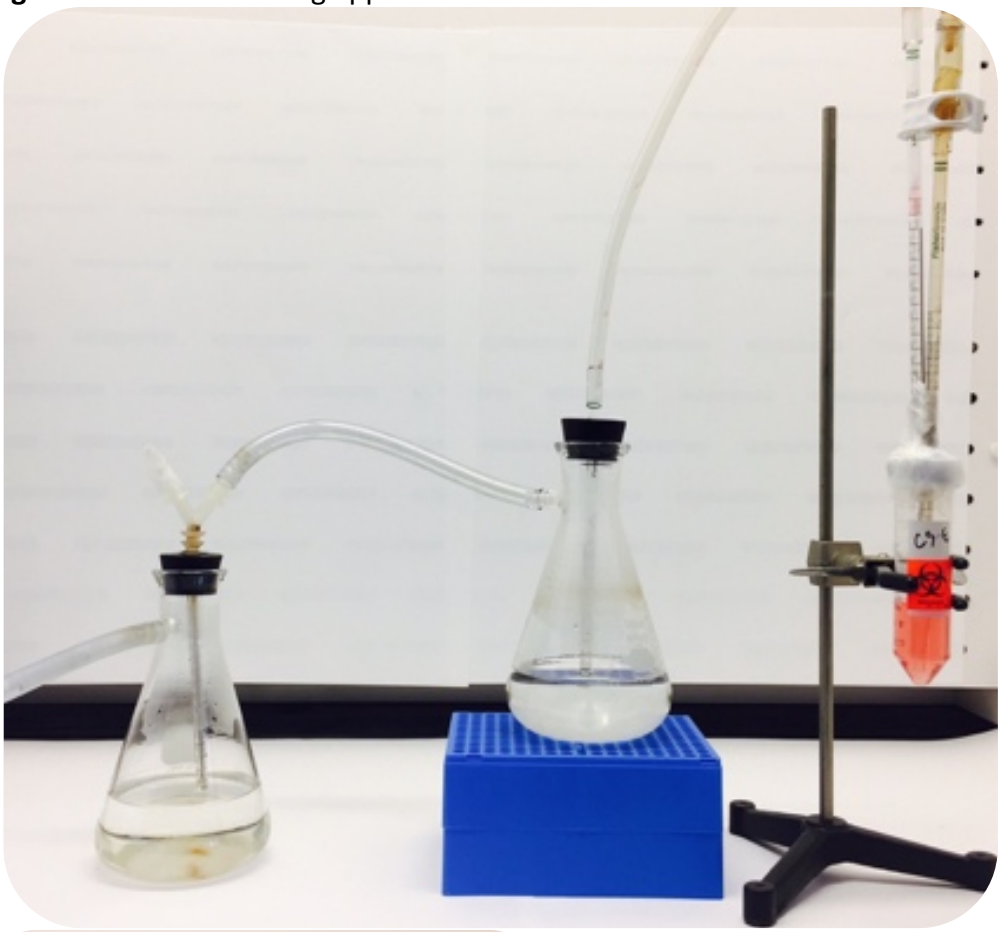


Figure 5. 48-well PCR plate testing 8 clinical biomarkers.

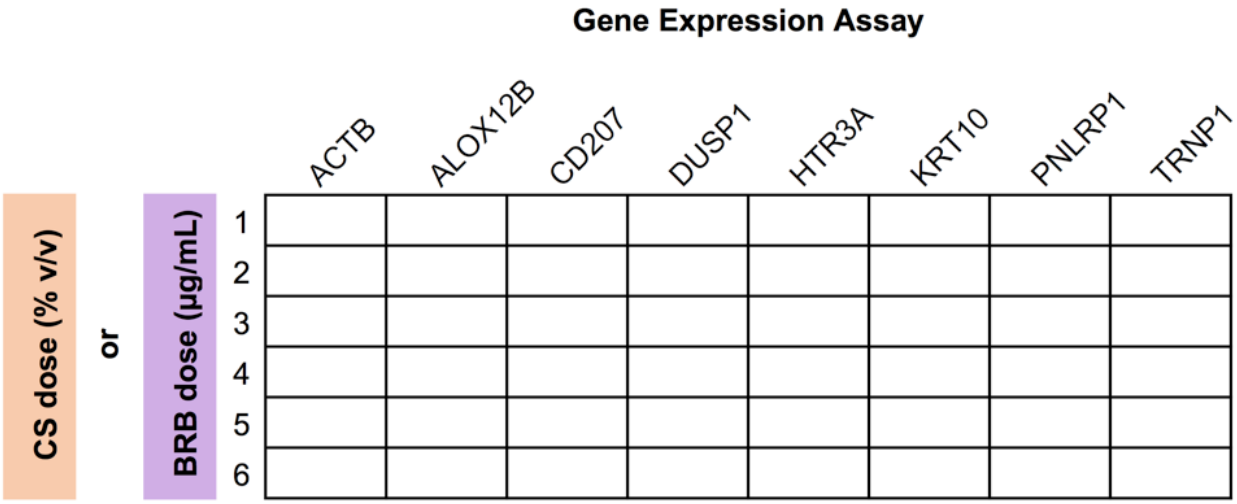


Figure 8. Human Cancer PathwayFinder Array Schema

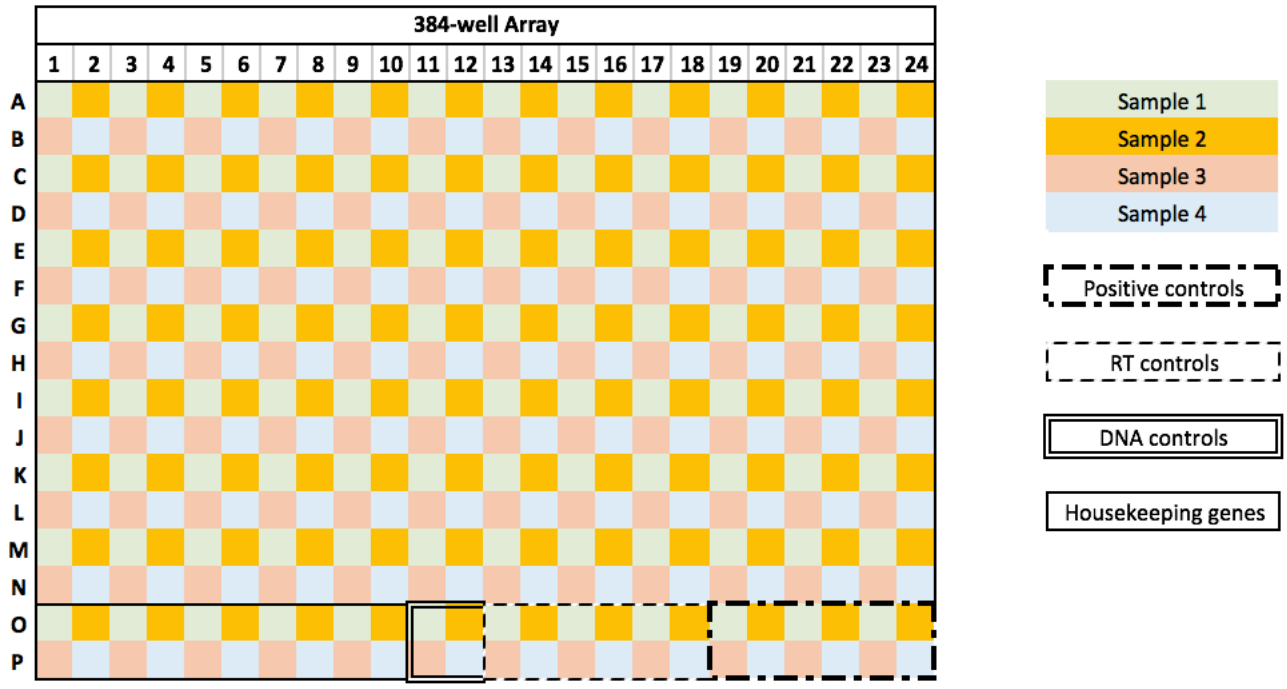


Figure 9. CS-E treated TE-1177 Network 1: *Cell Death and Survival, Organismal Injury and Abnormalities, Cardiovascular System Development and Function*

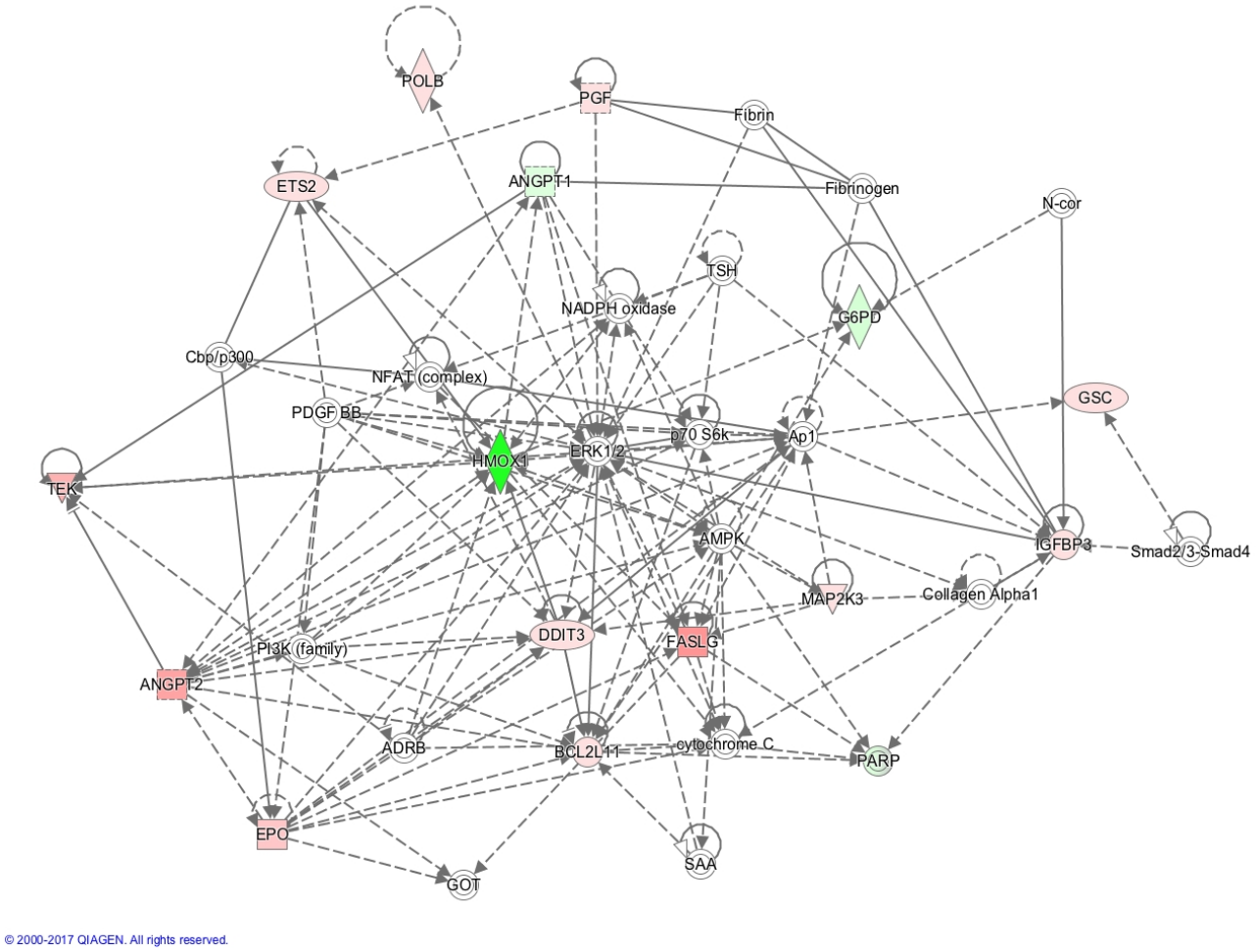


Figure 10: CS-E treated TE-1177 Network 2: *Developmental Disorder, Neurological Disease, Organismal Survival*

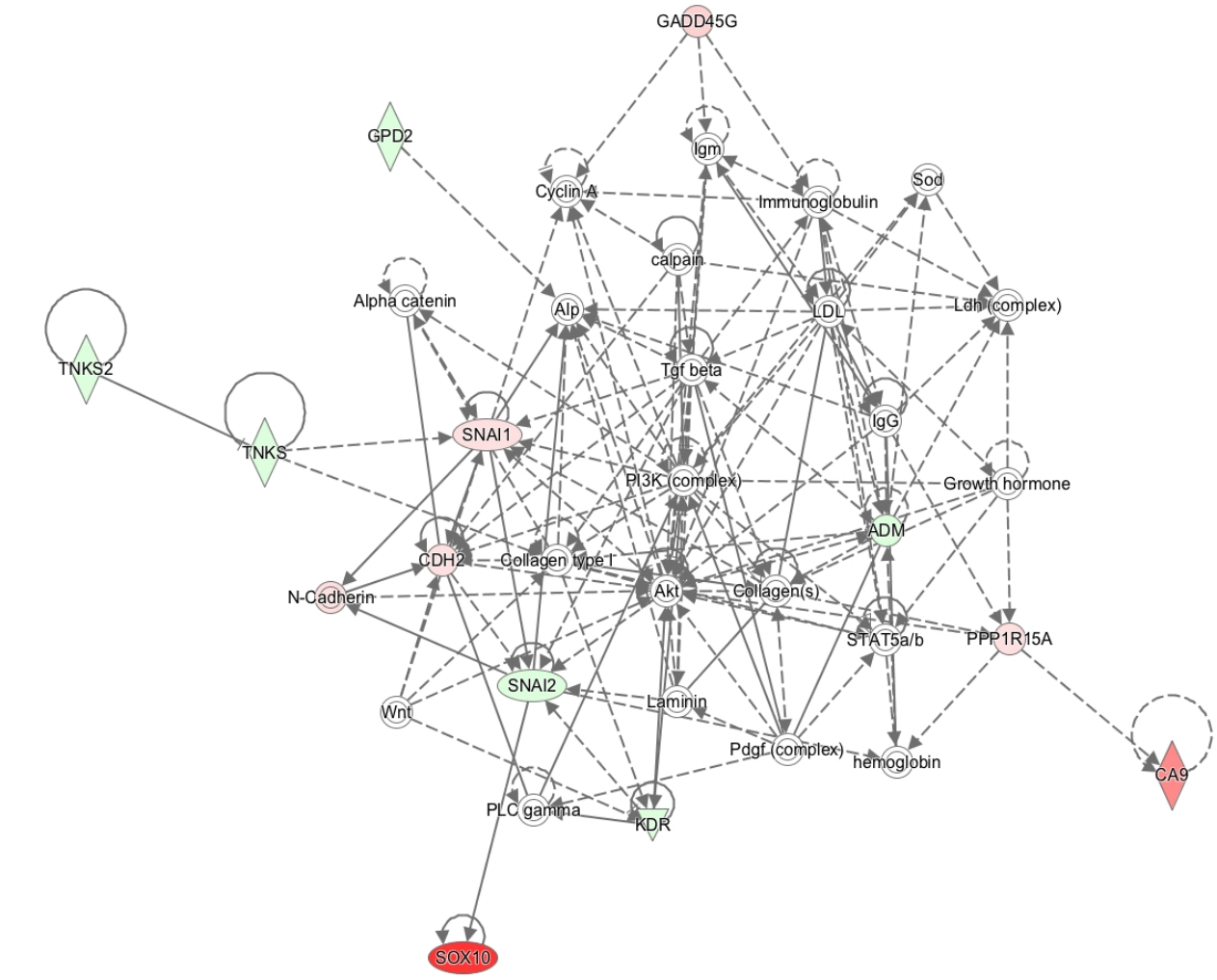


Figure 11: BRB- treated SCC-83 Network 4: *Cardiovascular System Development and Function, Cellular Movement, Connective Tissue Development and Function*

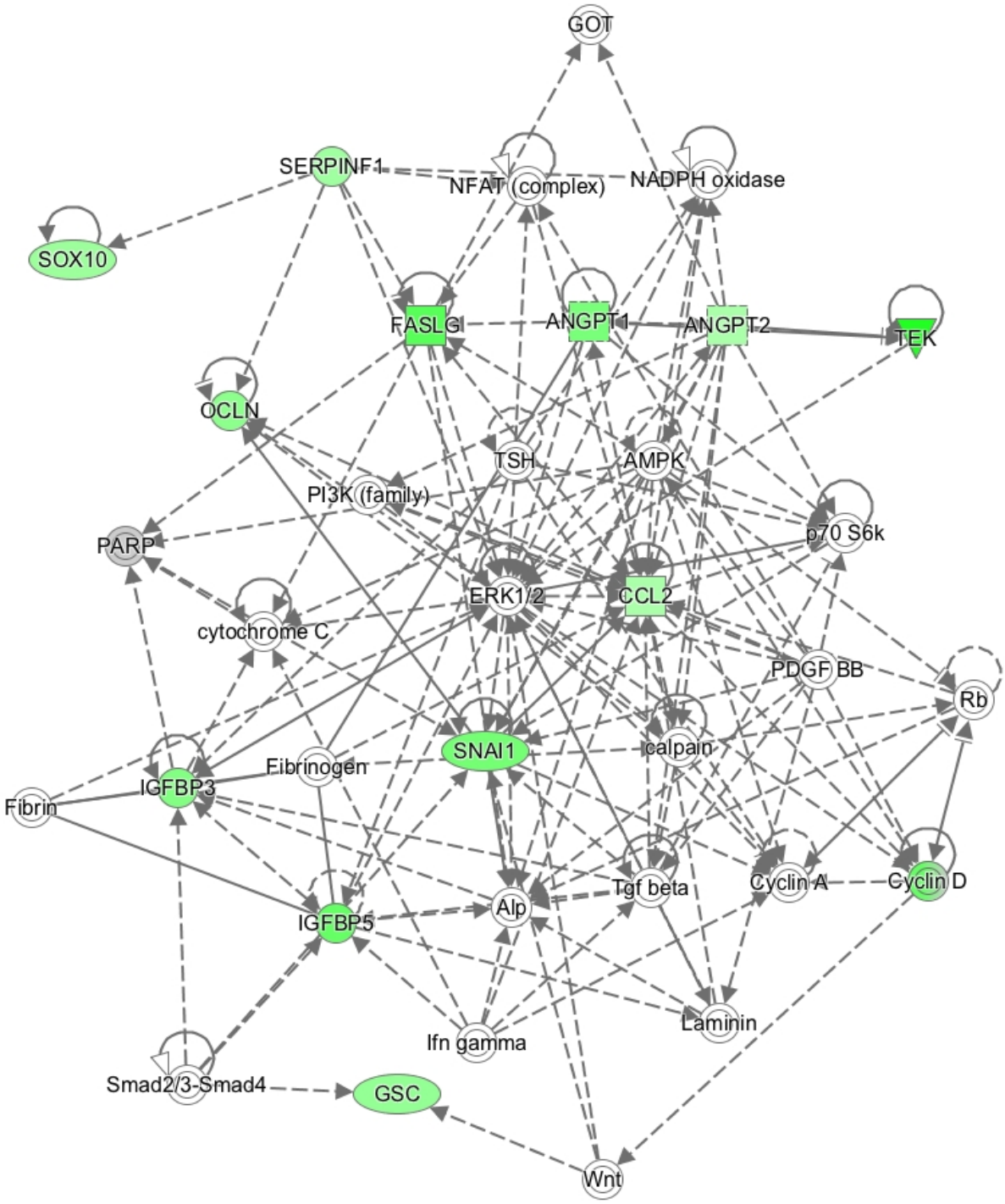


Figure 12: CS-E treated SCC-83 Network 8: *Cardiovascular System Development and Function, Organismal Development, Tissue Morphology*

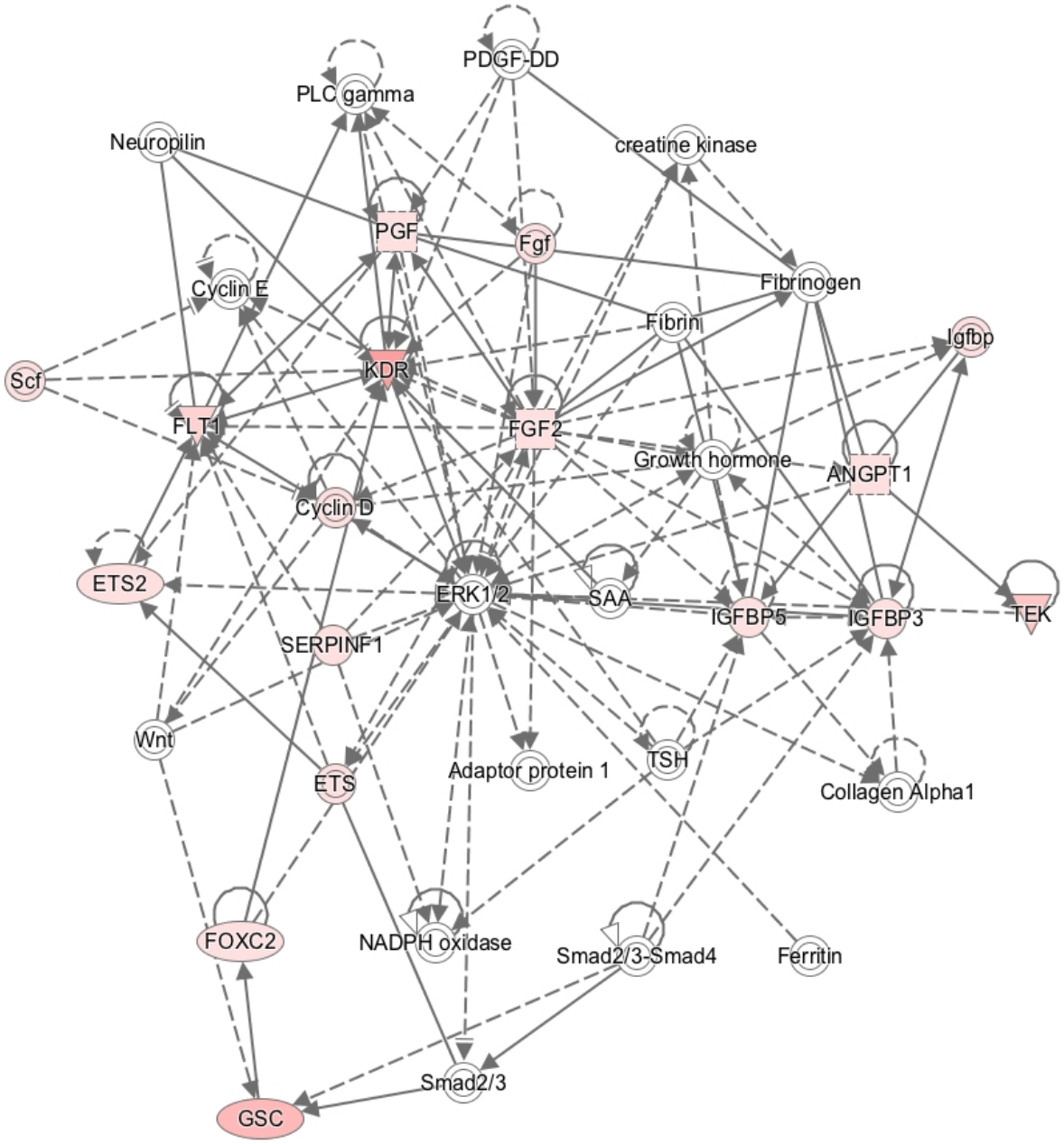


Figure 13: BRB-treated SCC-83 Network 5:

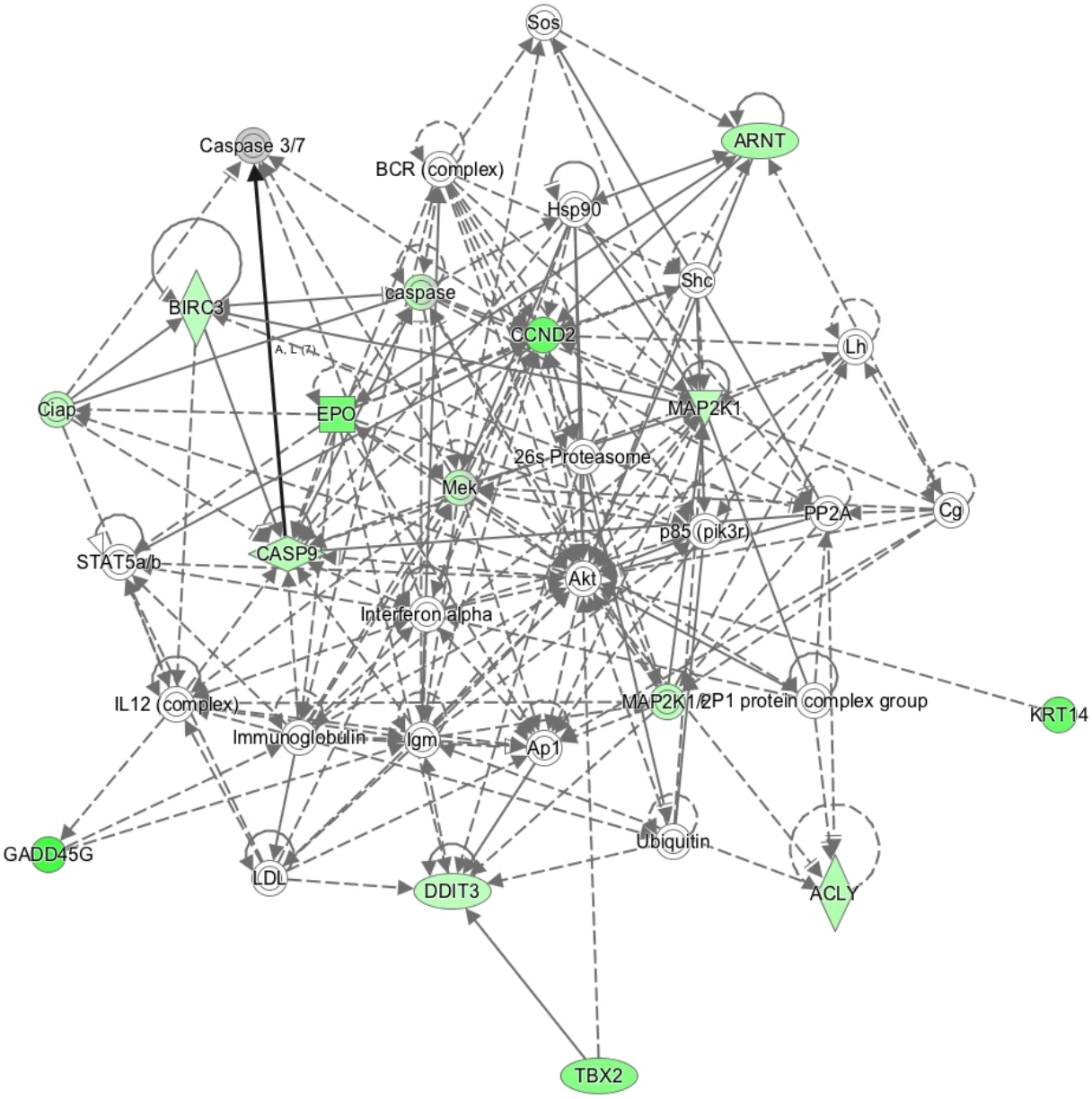
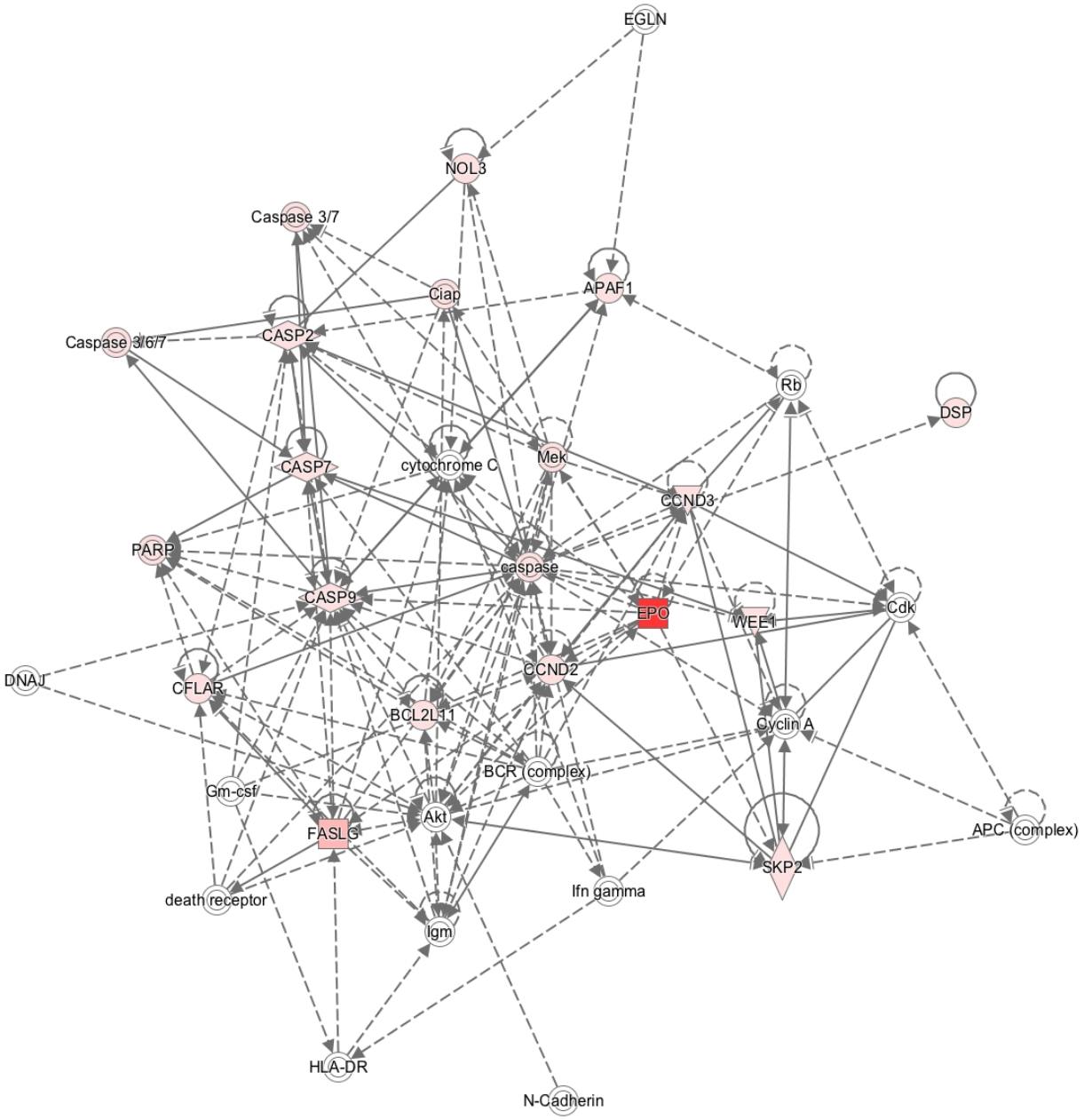


Figure 14: CS-E treated SCC-83 Network 7: *Cell Death and Survival, Cell Morphology, Cellular Function and Maintenance.*



APPENDIX

Table i. Sample RNA dilutions for CS-E treated cells.

SCC-83 SAMPLE	STOCK CONC ng/µL	STOCK VOL µL	TOTAL RNA ng	TARGET CONC1 ng/µL	+H2O µL	TARGET VOL1 µL	TARGET CONC2 ng/µL	TARGET1 VOL µL	+H2O µL	TARGET VOL2 µL
1–1	242.3	35.0	8480.5	100.0	49.8	84.8	20.0	7.0	28.0	35.0
1–2	181.0	35.0	6335.0	100.0	28.4	63.4	20.0	7.0	28.0	35.0
1–3	64.5	35.0	2257.5	50.0	10.2	45.2	20.0	14.0	21.0	35.0
1–4	10.7	35.0	374.5	•	•	•	20.0	35.0	0.0	35.0
1–5	17.8	35.0	623.0	•	•	•	20.0	35.0	0.0	35.0
1–6	22.1	35.0	773.5	•	•	•	20.0	35.0	3.3	38.3

SCC-83 SAMPLE	STOCK CONC ng/µL	STOCK VOL µL	TOTAL RNA ng	TARGET CONC1 ng/µL	+H2O µL	TARGET VOL1 µL	TARGET CONC2 ng/µL	TARGET1 VOL µL	+H2O µL	TARGET VOL2 µL
2–1	251.3	35.0	8795.5	100.0	53.0	88.0	20.0	7.0	28.0	35.0
2–2	183.7	35.0	6429.5	100.0	29.3	64.3	20.0	7.0	28.0	35.0
2–3	71.8	35.0	2513.0	50.0	15.3	50.3	20.0	14.0	21.0	35.0
2–4	13.0	35.0	455.0	•	•	•	20.0	35.0	0.0	35.0
2–5	12.3	35.0	430.5	•	•	•	20.0	35.0	0.0	35.0
2–6	20.9	35.0	731.5	•	•	•	20.0	35.0	1.5	36.5

CA-83 SAMPLE	STOCK CONC ng/µL	STOCK VOL µL	TOTAL RNA ng	TARGET CONC1 ng/µL	+H2O µL	TARGET VOL1 µL	TARGET CONC2 ng/µL	TARGET1 VOL µL	+H2O µL	TARGET VOL2 µL
3–1	286.9	35.0	10041.5	100.0	65.4	100.4	20.0	7.0	28.0	35.0
3–2	317.3	35.0	11105.5	100.0	76.1	111.1	20.0	7.0	28.0	35.0
3–3	23.8	35.0	833.0	•	•	•	20.0	35.0	0.0	35.0
3–4	4.0	35.0	140.0	•	•	•	20.0	35.0	0.0	35.0
3–5	10.1	35.0	353.5	•	•	•	20.0	35.0	0.0	35.0
3–6	6.0	35.0	210.0	•	•	•	20.0	35.0	0.0	35.0

CA-83 SAMPLE	STOCK CONC ng/µL	STOCK VOL µL	TOTAL RNA ng	TARGET CONC1 ng/µL	+H2O µL	TARGET VOL1 µL	TARGET CONC2 ng/µL	TARGET1 VOL µL	+H2O µL	TARGET VOL2 µL
4–1	322.1	35.0	11273.5	100.0	77.7	112.7	20.0	7.0	28.0	35.0
4–2	42.2	35.0	1477.0	•	•	•	20.0	35.0	0.0	35.0
4–3	26.3	35.0	920.5	•	•	•	20.0	35.0	8.4	43.4
4–4	24.7	35.0	864.5	•	•	•	20.0	35.0	6.7	41.7
4–5	16.6	35.0	581.0	•	•	•	20.0	35.0	0.0	35.0
4–6	20.2	35.0	707.0	•	•	•	20.0	35.0	0.3	35.3

TE-1177 SAMPLE	STOCK CONC ng/µL	STOCK VOL µL	TOTAL RNA ng	TARGET CONC1 ng/µL	+H2O µL	TARGET VOL1 µL	TARGET CONC2 ng/µL	TARGET1 VOL µL	+H2O µL	TARGET VOL2 µL
5–1	33.0	35.0	1155.0	20.0	•	•	20.0	35.0	13.8	48.8
5–2	19.4	35.0	679.0	20.0	•	•	20.0	35.0	0.0	35.0
5–3	43.0	35.0	1505.0	20.0	•	•	20.0	35.0	18.7	53.7
5–4	26.9	35.0	941.5	20.0	•	•	20.0	35.0	9.0	44.0
5–5	13.5	35.0	472.5	20.0	•	•	20.0	35.0	0.0	35.0
5–6	16.5	35.0	577.5	20.0	•	•	20.0	35.0	0.0	35.0

TE-1177 SAMPLE	STOCK CONC ng/µL	STOCK VOL µL	TOTAL RNA ng	TARGET CONC1 ng/µL	+H2O µL	TARGET VOL1 µL	TARGET CONC2 ng/µL	TARGET1 VOL µL	+H2O µL	TARGET VOL2 µL
6–1	29.4	35.0	1029.0	20.0	•	•	20.0	35.0	11.2	46.2
6–2	14.7	35.0	514.5	20.0	•	•	20.0	35.0	0.0	35.0
6–3	18.2	35.0	637.0	20.0	•	•	20.0	35.0	0.0	35.0
6–4	10.8	35.0	378.0	20.0	•	•	20.0	35.0	0.0	35.0
6–5	15.4	35.0	539.0	20.0	•	•	20.0	35.0	0.0	35.0
6–6	17.5	35.0	612.5	20.0	•	•	20.0	35.0	0.0	35.0

Table ii. Human Cancer PathwayFinder Sample Composition

Human Cancer PathwayFinder Microarray PCR - Sample Composition and Identification

Cell line + Treatment	Dose	EXP802: PCR Sample No.	PCR Plate No.	Experimental samples pooled [See Figure i. (appendix)]
TE1177 BRB-E	DMSO 800 µg/mL	1	1	4+10
	BRB 100 µg/mL	2	1	2+8
	BRB 200 µg/mL	3	1	3+9
	BRB 400 µg/mL	4	1	5+11
	BRB 800 µg/mL	5	2	6+12
TE1177 CS-E	CSE 0%	6	2	13+19
	CSE 1.25%	7	2	14+20
	CSE 2.5%	8	2	15+21
	CSE 5%	9	3	16+22
	CSE 10%	10	3	17+23
SCC83 BRB-E	DMSO 800 µg/mL	11	3	28+34
	BRB 100 µg/mL	12	3	26+32
	BRB 200 µg/mL	13	4	27+33
	BRB 400 µg/mL	14	4	29+35
	BRB 800 µg/mL	15	4	30+36
SCC83 CS-E	CSE 0%	16	4	37+43
	CSE 1.25%	17	5	38+44
	CSE 2.5%	18	5	39+45
	CSE 5%	19	5	40+46
	CSE 10%	20	5	41+47
CA83 BRB-E	DMSO 800 µg/mL	21	6	52+58
	BRB 100 µg/mL	22	6	50+56
	BRB 200 µg/mL	23	6	51+57
	BRB 400 µg/mL	24	6	53+59
	BRB 800 µg/mL	25	7	54+60
CA83 CS-E	CSE 0%	26	7	61+67
	CSE 1.25%	27	7	62+68
	CSE 2.5%	28	7	63+69
	CSE 5%	29	8	64+70
	CSE 10%	30	8	65+71

Table iii. Raw CT values for BRB-E treated TE177

Symbol	Control Group	BRB 100	BRB 200	BRB 400	BRB 800
ACLY	35	35	35	35	35
ACSL4	24.91	24.74	24.59	25.32	25.17
ADM	24.82	24.43	24.3	25.11	24.77
ANGPT1	27.48	27.15	27.25	28.16	28.39
ANGPT2	29.18	28.84	29.43	29.15	29.93
APAF1	27.32	26.83	27.06	27.34	27.53
ARNT	28.47	27.68	27.66	28.31	28.4
ATP5A1	22.99	22.79	22.86	23.28	23.31
AURKA	26.29	25.58	25.84	26.35	26.85
BCL2L11	28.94	27.73	28.89	28.66	29.12
BIRC3	28.63	28	28.17	28.55	28.9
BMI1	25.82	25.49	25.71	26.3	26.5
CA9	28.28	28.22	28.38	28.72	28.12
CASP2	27.53	27.37	27.6	27.78	28.21
CASP7	26.82	26.37	26.63	26.9	26.76
CASP9	27.62	27.24	27.23	27.45	27.58
CCL2	26.16	25.84	25.89	26.82	27.37
CCND2	26.75	26.36	26.61	27.18	27.23
CCND3	24.76	24.65	24.81	25.29	25.29
CDC20	26.7	25.93	26.4	26.74	27.29
CDH2	27.79	27.78	27.58	28.16	27.74
CFLAR	27.35	26.67	26.9	27.55	27.34
COX5A	24.6	24.25	24.19	24.41	24.63
CPT2	27.61	26.9	27.8	27.79	27.9
DDB2	26.76	26.17	26.37	26.58	26.91
DDIT3	27.59	27.3	27.13	27.44	27.41
DKC1	27.27	26.93	27.24	27.6	27.51
DSP	28.65	28.28	28.23	29.17	28.66
E2F4	26.64	25.94	26.31	26.8	26.79
EPO	28.85	28.42	27.87	28.81	29.39
ERCC3	27.38	26.95	27.36	27.38	27.36
ERCC5	26.48	26.43	26.39	26.96	26.64
ETS2	26.51	25.96	26.24	26.37	26.64
FASLG	28.75	27.94	28.65	29	29.51
FGF2	26.6	25.68	26.01	26.15	26
FLT1	28.32	27.58	27.88	28.82	28.95
FOXC2	26.94	26.69	26.81	27.27	26.94
G6PD	23.64	23.69	24.16	24.31	24.11
GADD45G	28.49	28.12	28.32	29.28	29.04
GPD2	26.56	25.89	26.4	26.61	27.13
GSC	29.33	29.24	29.36	29.16	29.69
HMOX1	27.07	26.65	27.1	27.1	26.72
IGFBP3	26.65	25.63	25.92	26.16	25.7
IGFBP5	25.72	24.89	25.34	25.89	25.89
IGFBP7	22.6	22.58	22.71	23.19	22.95
KDR	29.31	28.8	28.28	28.77	28.85
KRT14	27.53	26.86	26.76	27.46	27.66
LDHA	22.1	21.5	21.6	21.61	20.83
LIG4	27.33	26.88	27.14	27.53	27.79

LPL	28.69	28.45	28.53	29.11	29.58
MAP2K1	26.51	26.02	26.2	26.49	25.85
MAP2K3	27.66	27.23	27.43	27.3	27.57
MAPK14	27.47	27.21	27.32	27.76	27.61
MCM2	27.72	27.06	27.48	35	27.75
MKI67	27.66	26.66	27.16	27.6	27.67
NOL3	27.81	27.45	27.33	27.61	27.37
OCLN	27.91	27.25	27.91	28.34	28.44
PFKL	24.71	24.49	24.58	24.9	24.58
PGF	27.51	26.9	26.96	27.8	27.84
PINX1	28.51	27.3	27.85	27.92	27.94
POLB	27.75	27.41	27.6	27.77	27.87
PPP1R15A	27.32	26.71	26.87	27.36	26.88
SERPINB2	28.74	29	29.3	28.96	28.62
SERPINF1	24.02	23.91	23.93	24.58	24.41
SKP2	27.69	27.27	27.5	27.84	28.27
SLC2A1	27.51	27.19	26.99	27.02	26.47
SNAI1	29.22	29.57	29.76	29.71	30.27
SNAI2	24.33	24.16	24.43	24.79	24.75
SNAI3	27.99	27.65	28.14	27.96	28.13
SOD1	23.98	23.67	23.63	23.9	24
SOX10	28.87	28.11	28.53	29.65	29.18
STMN1	26.06	25.55	25.65	26.24	26.7
TBX2	26.75	26.17	26.19	26.58	26.96
TEK	28.57	27.68	27.49	27.71	28.23
TEP1	27.74	27.47	27.3	27.24	28.36
TERF1	26.91	26.63	26.78	27.39	27.43
TERF2IP	25.79	25.54	25.29	25.71	25.81
TINF2	27.42	26.5	26.94	27.01	27.44
TNKS	27.08	26.68	26.58	27.13	27.2
TNKS2	26.9	26.63	26.77	27.18	26.97
UQCRFS1	24.68	24.29	24.37	24.77	24.58
VEGFC	26.11	25.61	25.47	25.54	25.51
WEE1	27.62	27.17	27.19	27.03	27.86
XIAP	27.98	27.32	27.08	27.48	27.6
ACTB	19.37	18.95	18.85	19.31	18.96
B2M	21.99	21.94	21.98	22.47	21.96
GAPDH	19.63	19.42	19.31	19.49	18.83
HPRT1	27.36	26.89	26.82	27.17	27.19
RPLP0	20.44	20.09	20.01	20.38	20.24
HGDC	29.52	28.85	29.88	29.46	29.4
RTC	35	35	35	35	35
RTC	35	35	35	35	35
RTC	35	33.68	35	35	35
PPC	19.11	19.1	19.17	19.1	19.25
PPC	18.96	19.1	18.95	18.89	19.19
PPC	18.93	19.01	18.97	19.11	19.23

Table iv. *Raw CT values for CS-E treated TE-1177*

Symbol	**Control Group**	**CSE 1.25%**	**CSE 2.5%**	**CSE 5%**	**CSE 10%**
ACLY	35	35	35	35	35
ACSL4	24.93	24.95	25.22	27.3	26.3

ADM	24.75	22.48	23.69	25.84	24.71
ANGPT1	27.99	28.06	28.18	30.5	29.02
ANGPT2	34.57	30.98	30.84	31.56	30.5
APAF1	28.32	26.77	26.91	28.78	27.56
ARNT	29.5	28.96	28.81	29.83	28.88
ATP5A1	23.49	22.87	23.23	24.85	23.91
AURKA	27.2	25.11	25.42	26.92	26.18
BCL2L11	32.25	27.75	29.6	30.85	31.41
BIRC3	32.68	29.58	29.99	33.37	30.15
BMI1	25.68	25.46	26.11	27.71	27.32
CA9	33.95	28.61	28.49	30.57	29.13
CASP2	28.12	27.17	27.3	28.63	27.8
CASP7	27.17	26.73	27.14	28.76	27.27
CASP9	29.2	27.84	28.09	29.4	28.83
CCL2	26	26.34	27.1	27.68	27.26
CCND2	26.46	26.15	26.18	27.47	27.2
CCND3	24.15	23.55	23.68	25.65	24.74
CDC20	28.37	25.46	25.55	26.95	26.61
CDH2	28.43	26.09	26.37	28.19	27.25
CFLAR	26.61	26.19	26.14	27.78	26.73
COX5A	24	23.32	23.46	24.82	24.34
CPT2	28.92	28.41	28.43	30.13	29.27
DDB2	27.82	26.46	26.56	27.79	27.35
DDIT3	27.82	25.27	26.24	28.44	27.59
DKC1	27.1	26.6	26.79	28.89	28.08
DSP	29.71	27.2	27.44	29.56	28.29
E2F4	26.62	25.48	25.74	27.69	27
EPO	35	29.42	31.55	32.7	32.68
ERCC3	27.1	26.45	26.67	28.8	27.51
ERCC5	26.5	25.54	25.48	26.75	26.41
ETS2	26.8	24.69	25.02	27.52	26.47
FASLG	35	29.88	31.23	31.76	32.39
FGF2	25.42	24.79	24.9	26.94	25.27
FLT1	28.62	26.65	27.28	29.33	28.44
FOXC2	27.13	25.35	25.34	27.75	26.5
G6PD	22.87	24.35	24.73	25.92	25.16
GADD45G	32.13	24.5	28.78	30.16	29.91
GPD2	26.6	27.28	27.33	29.34	28.18
GSC	35	31.51	31.98	35	31.85
HMOX1	22.71	22.34	24.06	28.18	27.36
IGFBP3	22.81	21.15	21.15	22.65	21.97
IGFBP5	25.89	25.49	25.44	27.31	26.01
IGFBP7	21.98	20.79	20.84	22.18	21.83
KDR	32.91	30.56	30.9	35	31.82
KRT14	32.17	27.96	28.51	30.67	29.26
LDHA	21.3	19.85	20.07	21.7	20.88
LIG4	27.8	27.81	28.31	31.57	29.93
LPL	32.98	30.35	31.13	35	31.44
MAP2K1	25.9	25.15	25.2	27.56	26.27
MAP2K3	28.44	25.81	26.15	28.15	27.34
MAPK14	27.5	27.24	27.27	29.03	27.94
MCM2	28.68	27.46	27.64	29.69	28.91

MKI67	28.3	26.62	26.88	29.23	28
NOL3	27.78	26.98	27.62	29.34	27.73
OCLN	30.65	30.26	29.81	31.82	30.55
PFKL	24.74	23.91	23.98	25.98	24.99
PGF	28.94	26.68	27.76	29.96	29.36
PINX1	29.38	27.88	27.83	29.11	27.06
POLB	28.82	27.41	27.37	28.87	28.17
PPP1R15A	27.44	22.46	23.56	26.44	25.76
SERPINB2	28.59	26.87	27.84	29.31	28.87
SERPINF1	24.76	23.12	23.32	25.3	24.63
SKP2	27.96	27.5	27.71	29.19	28.58
SLC2A1	26.68	25.28	25.41	26.94	26.25
SNAI1	30.24	24.97	27.58	29.75	29.06
SNAI2	24.3	24.91	25.1	26.34	25.59
SNAI3	29.95	29.86	30.44	35	31.21
SOD1	23.77	22.77	22.97	24.77	23.88
SOX10	35	30.88	31.33	30.82	31.67
STMN1	26.68	24.61	24.51	25.9	25.64
TBX2	26.82	26.4	26.67	28.64	27.62
TEK	33.49	28.57	29.94	30.72	30.65
TEP1	29.24	28.73	28.76	30.17	29.46
TERF1	29.84	28.2	28.52	29.07	29.14
TERF2IP	25.44	24.76	24.83	26.87	26.46
TINF2	26.91	25.95	26.29	27.85	27.37
TNKS	26.99	26.85	27.55	29.73	28.28
TNKS2	27.44	26.52	27	29.57	27.85
UQCRFS1	24.45	23.69	24	25.82	24.88
VEGFC	25.45	24.41	24.65	26.84	25.6
WEE1	28.6	26.64	26.85	29.73	28.43
XIAP	28.25	27.32	27.49	29.36	28.41
ACTB	18.7	17.25	17.41	19.51	18.96
B2M	22.45	21.27	21.28	23	22.29
GAPDH	18.52	18.1	18.17	20.25	19.43
HPRT1	27.45	26.46	26.45	28.43	27.33
RPLP0	19.97	18.62	18.99	20.83	20.72
HGDC	35	30.63	30.76	35	31.33
RTC	35	35	35	35	35
RTC	35	35	35	35	35
RTC	35	35	35	35	35
PPC	19.34	19.39	19.28	19.33	19.18
PPC	19.4	19.4	19.27	19.26	19.2
PPC	19.36	19.3	19.12	19.27	19.23

Table v. Raw CT values for BRB-E treated SCC-83

Symbol	Control Group	BRB 100	BRB 200	BRB 400	BRB 800
ACLY	35	35	35	35	35
ACSL4	23.02	22.4	23.48	21.61	23.48
ADM	24.45	24.77	24.89	23.62	24.47
ANGPT1	27.29	27.76	27.5	28.08	27.44
ANGPT2	27.23	27.64	27.49	27.36	26.91
APAF1	25.27	24.93	25.6	24.09	25.21
ARNT	26.18	26.38	26.58	26.36	26.04
ATP5A1	22.41	22.25	22.94	21.14	22.97
AURKA	22.18	23.07	22.71	21.3	22.81
BCL2L11	25.76	25.93	26.3	25.38	25.78
BIRC3	25.35	25.23	25.66	25.14	25.46
BMI1	24.53	24.38	24.7	23.34	24.57
CA9	26.16	26.35	26.23	26.72	25.99
CASP2	23.91	23.96	24.6	23.17	24.46
CASP7	25.32	25.02	25.62	24.3	25.36
CASP9	25.51	25.73	25.86	25.47	25.7
CCL2	28.83	29.43	28.7	28.96	28.49
CCND2	26.61	27.34	27.13	27.61	26.73
CCND3	23.9	24.01	24.49	22.99	24.37
CDC20	22.4	22.76	23.27	21.89	23.62
CDH2	24.77	24.3	25.19	23.42	24.95
CFLAR	25.57	25.01	26.18	24.65	25.59
COX5A	22.71	22.36	23.2	21.6	22.9
CPT2	25.94	25.99	26.54	25.75	26.21
DDB2	25.36	25.3	25.75	24.47	25.32
DDIT3	25.9	26.18	26.32	25.69	25.85
DKC1	24.61	24.44	24.91	23.22	24.9
DSP	25.15	24.92	25.6	24.16	25.39
E2F4	23.67	23.47	24.29	22.53	24.15
EPO	26.47	27	26.7	27.34	26.54
ERCC3	24.98	24.63	25.47	23.8	25.23
ERCC5	24.92	24.95	25.51	24.27	25.21
ETS2	24.51	24.73	24.91	23.92	24.8
FASLG	26.53	27.19	26.99	27.64	26.68
FGF2	25.24	25.42	26.25	24.55	25.69
FLT1	27.18	27.16	27.31	28	26.64
FOXC2	25.31	24.89	25.75	24.35	25.63
G6PD	23.53	22.96	23.79	22.23	23.97
GADD45G	25.96	26.69	26.53	27.27	26.28
GPD2	25.24	25.34	25.6	24.54	25.55
GSC	28.85	29.53	28.21	29.32	28.67
HMOX1	25.49	25.91	26.28	25.09	25.52
IGFBP3	26.69	27.18	27	27.37	26.41
IGFBP5	25.75	25.5	26.18	26.74	25.96
IGFBP7	22.28	22.3	22.62	21.42	22.72
KDR	27.39	27.91	27.41	28.46	27.38
KRT14	25.15	25.83	25.44	26.16	25.17
LDHA	19.89	19.27	20.51	18.24	19.52
LIG4	25.2	25.28	25.75	24.85	25.5

LPL	27.4	28.03	27.57	28.38	27.38
MAP2K1	23.93	24.13	24.57	22.74	23.88
MAP2K3	25.63	25.85	26.61	25.42	25.96
MAPK14	26.3	25.99	26.84	25.1	26.73
MCM2	24.62	23.87	25.19	23.25	24.67
MKI67	22.25	21.96	22.85	21.27	22.94
NOL3	25.43	25.53	25.77	24.87	25.32
OCLN	25.38	25.95	25.93	25.95	25.77
PFKL	23.93	23.69	24.53	22.85	23.87
PGF	24.8	24.48	24.9	23.51	24.34
PINX1	26.41	26.71	26.38	25.79	26.18
POLB	25.44	25.57	25.86	24.5	25.42
PPP1R15A	24.45	24.58	25.3	23.74	24.54
SERPINB2	26.17	26.32	26.51	25.86	26.34
SERPINF1	25.95	26.16	26.37	26.25	26.14
SKP2	24.62	24.31	25.26	23.55	24.99
SLC2A1	23.88	23.46	24.55	22.43	23.8
SNAI1	28.41	29.1	28.46	29.26	28.24
SNAI2	24.86	24.6	25.24	23.42	24.71
SNAI3	27	27	27.55	27.49	27.15
SOD1	22.85	22.83	23.62	22.18	23.54
SOX10	26.76	26.69	26.66	27.13	26.02
STMN1	21.8	21.39	22.39	20.58	22.37
TBX2	26.63	26.88	26.77	27.27	26.19
TEK	25.36	25.99	26.01	26.9	25.53
TEP1	26.16	25.87	26.62	25.68	25.8
TERF1	24.77	24.85	25.25	24.9	24.71
TERF2IP	25.25	24.47	25.59	23.91	24.89
TINF2	25.25	24.72	25.73	24.26	25.51
TNKS	25.85	25.5	26.24	25.09	26.2
TNKS2	25.16	24.46	25.92	23.86	25.22
UQCRFS1	23.88	23.21	24.43	22.49	24.29
VEGFC	25.74	25.46	26.46	24.71	25.91
WEE1	24.64	24.14	25.49	23.85	24.9
XIAP	25.47	24.76	25.91	24.17	25.37
ACTB	17.33	16.53	18.17	15.69	17.73
B2M	20.5	20.2	20.92	19.15	20.86
GAPDH	18.24	17.89	18.85	16.89	18.22
HPRT1	23.76	23.79	24.46	22.71	24.19
RPLP0	19.83	19.39	20.58	18.39	20.14
HGDC	27.29	27.48	27.72	28.32	26.98
RTC	35	35	35	35	35
RTC	35	35	35	35	35
RTC	35	35	35	35	35
PPC	19.66	19.56	19.48	19.5	19.38
PPC	19.54	19.43	19.26	19.34	19.42
PPC	19.36	19.44	19.29	19.53	19.42

Table vi. Raw CT values for CS-E treated SCC-83

Symbol	Control Group	CSE 1.25%	CSE 2.5%	CSE 5%	CSE 10%
ACLY	15.121536	15.581391	15.267979	9.450422	9.388103
ACSL4	3.026712	3.042859	2.869168	2.955249	2.489997
ADM	4.429973	4.493207	2.377451	2.057718	2.112117
ANGPT1	9.069223	9.907651	12.525449	3.869444	3.427078
ANGPT2	11.782068	11.232569	10.204832	4.84143	3.82674
APAF1	5.683365	6.046788	6.046539	3.777076	2.824473
ARNT	7.902914	7.875453	8.467116	4.010315	2.65397
ATP5A1	2.021707	2.324465	2.414891	2.314022	1.990551
AURKA	2.299158	2.594399	2.386883	0.716697	0.393566
BCL2L11	5.947138	6.036215	5.689492	2.980455	2.687669
BIRC3	6.13344	6.437382	6.908072	3.210273	2.387525
BMI1	4.661243	4.816341	4.492937	3.725521	2.743345
CA9	11.019338	9.508411	9.160159	2.747858	1.901279
CASP2	4.939174	5.168612	5.02594	3.349058	3.299139
CASP7	5.352928	5.436415	5.395729	3.369257	3.500564
CASP9	6.765241	7.106551	6.720707	3.182121	2.246526
CCL2	15.121536	13.724835	15.267979	6.147516	4.931652
CCND2	10.451351	10.996476	10.846329	4.110784	2.776053
CCND3	4.789095	4.876223	4.218411	3.113552	3.203521
CDC20	3.336765	3.777066	3.133143	1.681505	1.234334
CDH2	4.544451	4.812689	5.059496	3.74842	3.619593
CFLAR	5.487699	5.547053	5.931489	3.726415	2.808153
COX5A	2.312402	2.482261	2.450302	1.875175	1.955535
CPT2	7.475981	6.906302	7.079214	4.32938	2.984485
DDB2	5.830377	6.067031	6.382321	3.391442	3.199787
DDIT3	7.970296	7.109112	4.634224	3.19055	3.256074
DKC1	4.795074	5.149249	4.502606	3.488371	3.528856
DSP	5.500519	5.71613	5.853411	3.373769	3.541131
E2F4	3.907041	4.229445	4.238907	3.339584	2.597438
EPO	15.121536	14.550091	13.079839	3.850216	3.055233
ERCC3	9.782449	5.206871	4.823998	3.08324	2.768903
ERCC5	5.453172	5.537875	5.736279	2.959789	3.055109
ETS2	4.612978	5.122708	4.697882	3.081859	3.019367
FASLG	13.422126	12.354088	14.679123	3.67688	2.89678
FGF2	5.460712	5.673342	5.916179	3.237014	3.205516
FLT1	12.706236	13.296071	14.423931	3.646234	3.120651
FOXC2	5.973421	6.030241	5.788668	2.75725	2.8712
G6PD	2.420713	2.314477	2.587017	3.96849	4.458936
GADD45G	12.093076	13.165671	12.263163	2.905517	2.778965
GPD2	5.586082	5.767872	6.01128	4.212012	3.820483
GSC	15.121536	15.581391	15.267979	5.41193	4.625398
HMOX1	5.060461	4.822558	2.819228	3.423959	2.63853
IGFBP3	8.418829	8.541526	7.947554	3.371727	3.027166
IGFBP5	8.060425	9.168854	8.736893	3.778507	2.629785
IGFBP7	1.787029	2.014134	2.491013	2.173637	1.810504
KDR	15.121536	15.581391	14.056819	4.924839	4.386753
KRT14	13.021416	11.35558	11.961626	2.207853	1.511087
LDHA	-0.343397	-0.143149	-0.077667	-0.269593	-0.376809
LIG4	5.891696	6.090302	5.598265	3.430835	2.172565

LPL	15.121536	14.132861	12.908955	4.71414	3.578445
MAP2K1	4.620323	4.598328	4.128365	3.392538	2.752584
MAP2K3	7.557104	7.485598	6.119819	3.940312	3.49473
MAPK14	6.108555	6.198876	6.507379	4.308489	3.871533
MCM2	4.440266	4.526482	4.525521	2.569128	2.325984
MKI67	3.080961	3.333831	3.547556	2.078146	2.268369
NOL3	5.763221	6.1386	6.244749	2.645562	2.348957
OCLN	8.978191	8.520367	8.395035	3.267082	2.093311
PFKL	3.228807	3.214345	4.238364	3.232677	2.697855
PGF	4.650232	5.03308	3.306525	3.198116	3.088005
PINX1	7.478317	7.795266	6.839596	4.735429	3.578025
POLB	5.749485	6.253756	5.710915	9.450422	3.253733
PPP1R15A	5.930313	6.700915	1.682375	2.161735	1.887575
SERPINB2	9.869188	7.351271	3.52232	4.629596	4.169076
SERPINF1	8.851198	9.186749	8.786684	3.930538	3.053283
SKP2	4.931019	5.098469	5.526529	3.248316	3.310472
SLC2A1	3.587351	3.850341	3.855593	2.976259	3.128318
SNAI1	13.800566	11.345036	4.703689	5.153873	4.927593
SNAI2	5.853349	5.524975	5.157999	2.841547	3.16271
SNAI3	9.443439	9.836884	9.633698	3.836477	3.381542
SOD1	2.846306	2.967509	2.453177	1.635992	1.292961
SOX10	12.814046	13.569641	12.868515	3.827131	3.204391
STMN1	1.391536	1.81453	1.857842	1.205026	1.450927
TBX2	10.101953	10.377352	9.377222	9.450422	5.116813
TEK	13.046506	12.429661	12.475849	3.632021	1.984113
TEP1	7.087826	7.242304	7.608111	4.246179	3.51526
TERF1	7.022028	8.117119	8.062401	2.103252	1.617223
TERF2IP	4.467866	4.610564	3.972157	3.626199	2.505432
TINF2	5.265253	5.06997	5.083098	3.015978	3.235387
TNKS	6.126978	6.153445	6.453543	4.319906	3.372421
TNKS2	4.909326	4.87613	5.906302	4.188337	2.902292
UQCRFS1	3.287903	3.187363	3.140656	2.815973	2.614263
VEGFC	6.236698	6.082049	5.846642	5.729349	4.335876
WEE1	4.593979	4.856963	4.858489	3.444702	2.55911
XIAP	4.977579	5.268981	5.677509	4.272478	3.279447
ACTB	-2.915871	-2.538039	-3.164021	9.450422	-2.252789
B2M	-0.671608	-1.082824	-0.42807	0.047367	0.637504
GAPDH	-2.146696	-1.859296	-2.126721	-2.122898	-2.374375
HPRT1	4.321499	4.446919	4.051394	3.445545	3.07962
RPLP0	-0.932869	-0.913383	-0.976789	-1.041395	-1.02497
HGDC	15.121536	13.825925	13.678784	4.056955	3.003925
RTC	15.121536	15.581391	15.267979	9.450422	9.388103
RTC	15.121536	15.581391	15.267979	9.450422	9.388103
RTC	15.121536	15.581391	15.267979	9.450422	9.388103
PPC	-0.446224	0.312401	0.021725	-6.16391	-6.061423
PPC	-0.523037	0.24252	-0.069452	-6.425268	-6.091136
PPC	-0.671464	0.198004	-0.031315	-6.285464	-6.179812

Table vii. Raw CT values for BRB-E treated CA-83

Symbol	Control Group	BRB 100	BRB 200	BRB 400	BRB 800
ACLY	35	35	35	35	35
ACSL4	22.46	22.6	28.5	28.1	21.74
ADM	23.91	22.11	27.61	27.72	21.45
ANGPT1	29.33	32.26	29.42	29.04	29.92
ANGPT2	30.65	29.94	30.39	29.44	29.21
APAF1	25.47	25.78	29.33	28.44	23.71
ARNT	27.29	28.2	29.56	28.27	26.94
ATP5A1	21.74	22.15	27.86	27.6	20.81
AURKA	22.01	22.12	26.27	26.01	21.12
BCL2L11	25.45	25.42	28.53	28.3	25.35
BIRC3	25.86	26.64	28.76	28	24.53
BMI1	24.23	24.22	29.28	28.36	22.79
CA9	28.93	28.89	28.3	27.51	28.43
CASP2	24.59	24.76	28.9	28.91	22.83
CASP7	24.86	25.13	28.92	29.11	24.37
CASP9	26.53	26.45	28.73	27.86	25.42
CCL2	33.14	35	31.7	30.54	32.41
CCND2	30.42	30.58	29.66	28.39	29.8
CCND3	24.29	23.95	20.63	20.57	22.74
CDC20	23.2	22.87	21.83	21.71	22.41
CDH2	24.23	24.79	22.62	22.79	22.82
CFLAR	24.97	25.66	31.69	30	24.27
COX5A	21.9	22.18	29.9	29.24	21.37
CPT2	26.32	26.81	24.63	24.49	25.66
DDB2	27.36	26.45	26.92	26.73	23.57
DDIT3	20.7	20.76	20.68	20.58	24.22
DKC1	20.57	20.49	20.55	18	23.3
DSP	25.47	25.54	25.46	25.45	23.98
E2F4	24.73	24.52	24.55	24.77	22.58
EPO	23.3	23.18	23.17	23.31	29.83
ERCC3	20.53	20.41	29.36	29.36	23.53
ERCC5	21.62	21.62	23.3	22.97	24.6
ETS2	22.74	22.77	24.83	24.8	23.77
FASLG	30.32	30.26	25.38	25.61	29.84
FGF2	29.49	29.27	32.86	32.18	24.57
FLT1	24.53	24.26	29.85	30.83	29.87
FOXC2	23.17	23.23	23.19	22.93	23.87
G6PD	21.83	21.75	21.74	21.56	21.9
GADD45G	23	23.1	23.14	23.19	29.52
GPD2	24.77	24.51	24.6	24.53	24.51
GSC	21.45	21.56	21.52	21.41	31.16
HMOX1	26.09	25.58	25.97	25.66	24.04
IGFBP3	29.17	29.31	24.54	24.35	26.16
IGFBP5	22.97	23.12	25.96	25.86	27.27
IGFBP7	24.72	24.6	23.62	23.49	21.29
KDR	25.47	25.45	24.47	24.32	31.09
KRT14	34.29	32.24	22.6	22.6	28.85
LDHA	30.74	29.77	30.61	30	16.76
LIG4	23.78	23.77	23.71	23.57	27.52

LPL	24.58	24.4	24.55	24.56	30.39
MAP2K1	24.35	24.22	24.23	24.16	22.1
MAP2K3	31.61	30.38	30.84	31.46	24.53
MAPK14	24.66	24.43	24.42	24.57	24.71
MCM2	31.28	30.43	30.93	30.13	22.5
MKI67	24.37	23.86	23.79	23.8	21.47
NOL3	26.13	25.65	22.19	21.71	24.34
OCLN	23.3	23.12	30.99	30.37	25.65
PFKL	24.23	24.35	24.93	24.81	22.31
PGF	22.62	22.66	32.25	32.32	23.81
PINX1	31.36	30.78	24.95	24.96	25.73
POLB	26.89	26.94	27.26	26.97	23.82
PPP1R15A	26.4	24.32	24.42	25.41	21.8
SERPINB2	20.82	20.66	20.7	21.22	25.42
SERPINF1	30.86	30.17	33.6	30.44	26.12
SKP2	29.39	28.85	30.17	28.58	23.24
SLC2A1	18.22	17.94	17.97	17.71	21.48
SNAI1	23.88	23.88	25.25	24.91	29.86
SNAI2	22.2	21.85	31.66	30.57	23.69
SNAI3	30.21	29.66	23.01	23.01	27.84
SOD1	24.67	24.56	24.98	24.98	21.74
SOX10	35	31.57	25.11	25.01	28.78
STMN1	24.92	24.99	23.02	22.71	20.68
TBX2	21.49	21.31	21.21	21.31	27.35
TEK	24.69	24.82	24.65	24.57	29.26
TEP1	26.88	26.51	26.78	26.05	25.7
TERF1	22.92	22.96	23.12	22.69	25.44
TERF2IP	24.56	24.46	24.34	23.95	23.61
TINF2	25.94	24.63	26.13	25.85	24.14
TNKS	25	24.86	24.22	24.21	24.96
TNKS2	31.48	30.86	23.5	23.38	23.7
UQCRFS1	22.96	22.62	27.85	27.03	22.01
VEGFC	24.95	24.84	26.52	26.55	23.98
WEE1	24.99	24.77	23.24	23.32	23.42
XIAP	22.75	22.56	22.4	22.29	24.65
ACTB	31.28	30.9	31.21	30.89	15.59
B2M	24.62	24.48	24.6	23.67	19.54
GAPDH	28.21	28.31	28.19	27.69	16.2
HPRT1	21.7	21.72	21.69	21.68	22.32
RPLP0	29.21	29.31	30.83	30.23	17.72
HGDC	20.69	20.5	20.54	20.58	29.68
RTC	24.24	24.1	27.67	27.62	35
RTC	23.47	23.48	29.88	29.85	35
RTC	27.43	27.49	25.98	26.14	35
PPC	26.55	26.35	25.49	25.63	19.7
PPC	23.35	23.13	24.24	23.97	19.53
PPC	22.24	22.22	24.31	24.27	19.59

Table vii. Raw CT values for CS-E treated CA-83

Symbol	Control Group	CSE 1.25%	CSE 2.5%	CSE 5%	CSE 10%
ACLY	35	35	35	35	35
ACSL4	21.92	22.61	23.65	28.21	29.39
ADM	22.39	20.37	20.16	25.12	27.45
ANGPT1	30.45	29.85	34.21	30.01	30.61
ANGPT2	30.4	30.77	31.31	32.69	30.88
APAF1	24.53	25.63	26.49	28.88	30.82
ARNT	26.82	27.23	29.64	30.78	30.15
ATP5A1	20.67	21.61	23.39	26.47	27.64
AURKA	20.69	21.74	22.8	25.02	27.35
BCL2L11	25.23	24.81	24.85	27.74	29.5
BIRC3	24.14	25.17	28.16	29.31	29.96
BMI1	23.53	23.99	24.93	28.99	30.68
CA9	32.58	28.27	28.35	29.15	30.38
CASP2	23.7	24.35	25.62	28.46	29.68
CASP7	24.67	25.01	26.17	28.69	29.67
CASP9	25.24	25.78	27.18	29.54	30.39
CCL2	34.3	32.35	35	31.41	32.22
CCND2	31.92	31.33	31.55	30.31	30.7
CCND3	23.44	23.84	24.55	27.37	29.53
CDC20	22	23.13	23.87	26.26	27.97
CDH2	23.11	23.89	25.51	28.61	30.29
CFLAR	24.08	24.69	26.82	28.32	30.47
COX5A	21.36	21.88	23.21	25.95	27.43
CPT2	25.72	26.21	29.34	29.92	30.65
DDB2	24.37	25.18	26.59	28.59	28.94
DDIT3	26.5	23.77	23.52	28.29	30.48
DKC1	23.67	24.48	25.14	28.47	29.36
DSP	24.39	24.89	26.48	30.2	31.17
E2F4	23.11	23.75	24.93	27.57	29.43
EPO	32.63	32.1	33.61	30.6	30.69
ERCC3	23.72	24.65	25.65	28.61	30.2
ERCC5	24.39	25.16	26.86	28.77	29.66
ETS2	24.49	24.5	24.19	28.62	30.79
FASLG	31.59	30.72	34.34	30.12	31.83
FGF2	24.65	25.16	26.36	28.53	30.13
FLT1	35	30.72	31.89	29.74	30.24
FOXC2	24.16	24.93	25.86	27.39	29.47
G6PD	21.4	22.09	24.2	27.5	29.06
GADD45G	33.41	29.94	31.92	29.76	30.35
GPD2	24.79	25.59	27.15	29.64	30.81
GSC	35	32.58	33.18	31.42	31.61
HMOX1	23.9	22.16	22.06	28.58	30.09
IGFBP3	27.36	26.24	26.39	29.32	30.54
IGFBP5	24.68	25.46	27.41	28.96	30.27
IGFBP7	19.92	20.28	22.38	25.17	26.76
KDR	35	31.55	35	30.32	30.47
KRT14	31.31	29.03	30.82	29.45	28.96
LDHA	18.15	19.08	20.72	23.9	25.18

LIG4	24.93	25.54	26.24	29.49	29.97
LPL	35	31.87	32.99	31	33.48
MAP2K1	23.29	23.89	24.65	28.26	29.81
MAP2K3	26.33	25.76	25.33	29.57	30.67
MAPK14	25.37	26.2	27.88	29.94	31.25
MCM2	23.18	24.21	25.22	28.6	29.3
MKI67	21.63	23.29	24.59	26.62	28.65
NOL3	24.58	24.96	26.94	28.43	29.23
OCLN	26.94	27.58	29.75	29.32	29.23
PFKL	22.61	23.12	25.09	28.12	29.48
PGF	23.18	22.78	22.15	27.72	29.87
PINX1	25.85	26.41	27.62	29.48	30.45
POLB	24.48	24.83	25.84	28.53	30.11
PPP1R15A	23.62	21.11	19.91	25.99	28.4
SERPINB2	28.94	27.69	26.16	30.25	30.57
SERPINF1	26.94	27.32	28.67	29.58	30
SKP2	23.92	24.95	26.88	28.88	30.16
SLC2A1	22.17	22.75	24.67	27.39	29.55
SNAI1	32.06	23.36	22.57	29.61	32.3
SNAI2	25.41	25.68	26.85	29.48	29.95
SNAI3	28.68	28.43	30.19	31.4	30.9
SOD1	22	22.23	22.76	26.57	27.98
SOX10	30.85	29.63	32.16	30.28	31.58
STMN1	20.48	21.25	21.96	24.89	27.04
TBX2	28.45	28.81	28.46	29.36	29.82
TEK	35	29.79	31.8	28.55	29.71
TEP1	25.69	25.41	27.13	29.68	33.19
TERF1	26.49	26.56	27.69	28.19	29.36
TERF2IP	23.37	23.53	24.9	28	29.3
TINF2	23.92	24.4	25.34	28.14	29.22
TNKS	25.1	25.88	27.55	30.41	31.76
TNKS2	23.91	24.76	26.22	28.9	31.65
UQCRFS1	22.12	22.83	24.54	27.39	28.45
VEGFC	24.19	24.68	26.47	29.24	31.7
WEE1	23.35	23.92	24.6	27.91	28.83
XIAP	24.52	25.19	27.2	29.87	30.15
ACTB	16.13	16.49	17.45	20.64	22.13
B2M	18.69	18.7	20.69	23.36	25.36
GAPDH	16.94	17.41	18.52	21.79	23.24
HPRT1	23.16	23.63	24.41	27.67	29.37
RPLP0	18.23	18.65	19.69	23.31	24.39
HGDC	34.14	30.94	31.85	30.33	30.7
RTC	35	35	35	35	35
RTC	35	35	35	35	35
RTC	35	35	35	35	35
PPC	19.77	19.77	19.62	19.94	19.87
PPC	19.63	19.83	19.54	19.82	19.82
PPC	19.7	20.57	19.71	19.76	19.63

Table viii. Fold-changes for BRB-E treated TE-1177 compared to control group.

		BRB-100		BRB-200		BRB-400	
Position	**Symbol**	**Fold Change**	**Comments**	**Fold Change**	**Comments**	**Fold Change**	**Comments**
A01	ACLY	0.8333	C	0.8008	C	1.017	C
A02	ACSL4	0.9326		0.9932		0.7605	
A03	ADM	1.0887		1.1411		0.8301	
A04	ANGPT1	1.0448		0.9398		0.6353	
A05	ANGPT2	1.0568		0.6758		1.0383	
A06	APAF1	1.1771		0.9589		1.0085	
A07	ARNT	1.4451		1.4056		1.1332	
A08	ATP5A1	0.9541		0.8771		0.8299	
A09	AURKA	1.3656		1.0915		0.9762	
A10	BCL2L11	1.9251		0.8295		1.2327	
A11	BIRC3	1.2863		1.0958		1.071	
A12	BMI1	1.0512		0.87		0.7299	
B01	CA9	0.8688		0.7443		0.7478	
B02	CASP2	0.9318		0.7621		0.8516	
B03	CASP7	1.1375		0.9135		0.9638	
B04	CASP9	1.083		1.0539		1.149	
B05	CCL2	1.0436		0.9681		0.6471	
B06	CCND2	1.0907		0.8823		0.7546	
B07	CCND3	0.9015		0.7739		0.7051	
B08	CDC20	1.4183		0.987		0.9933	
B09	CDH2	0.8381		0.9307		0.7905	
B10	CFLAR	1.3362		1.097		0.8843	
B11	COX5A	1.0618		1.0675		1.1633	
B12	CPT2	1.367		0.7014		0.8995	
C01	DDB2	1.248		1.0449		1.1485	
C02	DDIT3	1.0224		1.1051		1.1322	
C03	DKC1	1.0585		0.82		0.81	
C04	DSP	1.0784		1.0736		0.71	
C05	E2F4	1.3518		1.0042		0.9061	
C06	EPO	1.1227		1.5721		1.044	
C07	ERCC3	1.1209		0.8104		1.0196	
C08	ERCC5	0.8607		0.8484		0.7252	
C09	ETS2	1.2158		0.9647		1.1154	
C10	FASLG	1.464		0.8589		0.8586	
C11	FGF2	1.5774		1.212		1.3911	
C12	FLT1	1.3878		1.0893		0.7179	
D01	FOXC2	0.993		0.8788		0.8118	
D02	G6PD	0.8039		0.5592		0.6394	
D03	GADD45G	1.0724		0.9022		0.5852	
D04	GPD2	1.3276		0.8981		0.9839	
D05	GSC	0.8871		0.7828		1.1394	
D06	HMOX1	1.1131		0.7801		0.9904	
D07	IGFBP3	1.6826		1.3281		1.4252	
D08	IGFBP5	1.487		1.0431		0.9063	
D09	IGFBP7	0.8465		0.7432		0.6739	
D10	KDR	1.1865		1.6371		1.4751	
D11	KRT14	1.3272		1.3708		1.069	
D12	LDHA	1.2626		1.1303		1.4292	

E01	LIG4	1.1418		0.9117		0.8835	
E02	LPL	0.9805		0.8931		0.7617	
E03	MAP2K1	1.167		0.9955		1.0336	
E04	MAP2K3	1.12		0.938		1.2992	
E05	MAPK14	1.0028		0.8876		0.8323	
E06	MCM2	1.3189		0.9457		0.0065	A
E07	MKI67	1.6695		1.135		1.058	
E08	NOL3	1.0706		1.1149		1.17	
E09	OCLN	1.3166		0.8011		0.7526	
E10	PFKL	0.9709		0.8757		0.8961	
E11	PGF	1.2707		1.1733		0.8327	
E12	PINX1	1.9263		1.2618		1.5314	
F01	POLB	1.0506		0.8876		1.0019	
F02	PPP1R15A	1.2713		1.0918		0.9847	
F03	SERPINB2	0.6979		0.5436		0.874	
F04	SERPINF1	0.8979		0.8533		0.6904	
F05	SKP2	1.1158		0.9158		0.9142	
F06	SLC2A1	1.0451		1.1534		1.4342	
F07	SNAI1	0.6539		0.5498		0.7269	
F08	SNAI2	0.9345		0.7457		0.7386	
F09	SNAI3	1.0534		0.7204		1.0389	
F10	SOD1	1.0311		1.0176		1.0681	
F11	SOX10	1.4069		1.014		0.5895	
F12	STMN1	1.1889		1.0614		0.895	
G01	TBX2	1.2444		1.1867		1.1451	
G02	TEK	1.5506		1.6985		1.8491	
G03	TEP1	1.0031		1.0883		1.4416	
G04	TERF1	1.0092		0.8761		0.7283	
G05	TERF2IP	0.9898		1.1333		1.0758	
G06	TINF2	1.5743		1.1167		1.358	
G07	TNKS	1.1021		1.1292		0.9822	
G08	TNKS2	1.0075		0.8742		0.8391	
G09	UQCRFS1	1.0881		0.9929		0.9536	
G10	VEGFC	1.1736		1.2437		1.501	
G11	WEE1	1.1368		1.0788		1.534	
G12	XIAP	1.3107		1.4909		1.4343	
H01	ACTB	1.1171		1.1499		1.0612	
H02	B2M	0.8597		0.8043		0.7257	
H03	GAPDH	0.9645		1.0019		1.1254	
H04	HPRT1	1.1563		1.161		1.1603	
H05	RPLP0	1.0652		1.0839		1.0621	
H06	HGDC	1.3282		0.6228		1.0569	
H07	RTC	0.8333	C	0.8008	C	1.017	C
H08	RTC	0.8333	C	0.8008	C	1.017	C
H09	RTC	2.0818	B	0.8008	C	1.017	C
H10	PPC	0.8388		0.7681		1.0175	
H11	PPC	0.7551		0.8035		1.0656	
H12	PPC	0.7862		0.7749		0.8971	

Table ix. Fold-changes for CS-E treated TE-1177 compared to control group.

		1.25% CSE		2.5% CS-E		5% CS-E	
Position	Symbol	Fold Regulation	Comments	Fold Regulation	Comments	Fold Regulation	Comments
A01	ACLY	-1.9725	C	-1.8108	C	2.0956	C
A02	ACSL4	-2.0012		-2.219		-2.4728	
A03	ADM	2.4415		1.1487		-1.0154	
A04	ANGPT1	-2.0668		-2.0629		-2.7168	A
A05	ANGPT2	6.1236	B	7.3196	B	16.8706	B
A06	APAF1	1.4853		1.4674		1.5165	
A07	ARNT	-1.3572		-1.1226		1.6625	
A08	ATP5A1	-1.2778		-1.5065		-1.2192	
A09	AURKA	2.1629		1.9032		2.5448	
A10	BCL2L11	11.4247	A	3.4602	A	5.5364	B
A11	BIRC3	4.3342	A	3.5553	A	1.2974	B
A12	BMI1	-1.6945		-2.4459		-1.9508	
B01	CA9	20.4503	A	24.3464	A	21.7789	B
B02	CASP2	-1.023		-1.0285		1.467	
B03	CASP7	-1.4561		-1.7742		-1.4354	
B04	CASP9	1.3037		1.1907		1.8214	
B05	CCL2	-2.5095		-3.8964		-1.5334	
B06	CCND2	-1.5938		-1.4943		1.0376	
B07	CCND3	-1.3026		-1.3113		-1.35	
B08	CDC20	3.8064		3.8902		5.5997	
B09	CDH2	2.5768		2.3122		2.4878	
B10	CFLAR	-1.467		-1.3019		-1.0712	
B11	COX5A	-1.2312		-1.2452		1.1912	
B12	CPT2	-1.3851		-1.2894		-1.1071	A
C01	DDB2	1.3084		1.3287		2.15	
C02	DDIT3	2.9675		1.6497		1.3606	
C03	DKC1	-1.3927		-1.46		-1.6442	
C04	DSP	2.8886		2.6635		2.3279	
C05	E2F4	1.1239		1.0229		-1.0009	
C06	EPO	24.24	A	6.0251	B	10.3181	B
C07	ERCC3	-1.2562		-1.3441		-1.5501	
C08	ERCC5	-1.0113		1.1189		1.7696	
C09	ETS2	2.197		1.9013		1.2732	
C10	FASLG	17.6165	A	7.5448	B	19.7412	B
C11	FGF2	-1.2783		-1.267		-1.3718	
C12	FLT1	1.9858		1.3985		1.2805	
D01	FOXC2	1.744		1.906		1.36	
D02	G6PD	-5.5251		-6.5827		-3.9685	
D03	GADD45G	100.7028	A	5.6101	A	8.2248	B
D04	GPD2	-3.1707		-3.0066		-3.1908	
D05	GSC	5.7094	B	4.4906	B	2.0956	C
D06	HMOX1	-1.5282		-4.6254		-21.1017	
D07	IGFBP3	1.5965		1.7379		2.3397	
D08	IGFBP5	-1.4912		-1.3208		-1.2689	
D09	IGFBP7	1.1605		1.2169		1.8308	
D10	KDR	2.5907	B	2.2279	B	-2.0284	B
D11	KRT14	9.3929	A	7.0156	A	5.9441	B

D12	LDHA	1.3861		1.2967		1.5873	
E01	LIG4	-1.9833		-2.5776		-6.49	A
E02	LPL	3.1544	B	1.9945	B	-1.9309	B
E03	MAP2K1	-1.1696		-1.1147		-1.5055	
E04	MAP2K3	3.1389		2.6938		2.5597	
E05	MAPK14	-1.6438		-1.5382		-1.372	
E06	MCM2	1.1839		1.1424		1.0428	
E07	MKI67	1.6248		1.472		1.099	
E08	NOL3	-1.1309		-1.6206		-1.4083	
E09	OCLN	-1.5084	B	-1.0174	A	-1.0733	B
E10	PFKL	-1.1121		-1.0693		-1.13	
E11	PGF	2.4294		1.2514		1.0341	
E12	PINX1	1.4363		1.6201		2.5174	
F01	POLB	1.3507		1.5084		2.0182	
F02	PPP1R15A	16.0162		8.1748		4.2122	
F03	SERPINB2	1.6674		-1.0764		1.2709	
F04	SERPINF1	1.5867		1.4975		1.4464	
F05	SKP2	-1.4259		-1.5137		-1.1146	
F06	SLC2A1	1.3423		1.3305		1.749	
F07	SNAI1	19.6617	A	3.4877	A	2.9463	A
F08	SNAI2	-2.9981		-3.1436		-1.9544	
F09	SNAI3	-1.858		-2.5486	A	-15.8604	A
F10	SOD1	1.0141		-1.0447		1.0429	
F11	SOX10	8.8412	B	7.0085	B	38.1083	B
F12	STMN1	2.1304		2.4715		3.5801	
G01	TBX2	-1.4773		-1.6328		-1.686	
G02	TEK	15.3241	A	6.4717	A	14.2962	B
G03	TEP1	-1.3869		-1.2985		1.0996	A
G04	TERF1	1.5805		1.3807		3.5701	
G05	TERF2IP	-1.2288		-1.1853		-1.2884	
G06	TINF2	-1.0145		-1.1772		1.0901	
G07	TNKS	-1.783		-2.6647		-3.1727	
G08	TNKS2	-1.0451		-1.3339		-2.0858	
G09	UQCRFS1	-1.1608		-1.3233		-1.2289	
G10	VEGFC	1.041		-1.0401		-1.252	
G11	WEE1	1.9776		1.8531		-1.0472	
G12	XIAP	-1.035		-1.0669		-1.0327	
H01	ACTB	1.3856		1.3571		1.1981	
H02	B2M	1.1467		1.2373		1.4303	
H03	GAPDH	-1.4766		-1.4181		-1.5887	
H04	HPRT1	1.0098		1.1074		1.0651	
H05	RPLP0	1.2921		1.0885		1.157	
H06	HGDC	10.5112	B	10.4566	B	2.0956	C
H07	RTC	-1.9725	C	-1.8108	C	2.0956	C
H08	RTC	-1.9725	C	-1.8108	C	2.0956	C
H09	RTC	-1.9725	C	-1.8108	C	2.0956	C
H10	PPC	-2.0345		-1.7351		2.1138	
H11	PPC	-1.9752		-1.6627		2.3048	
H12	PPC	-1.8912		-1.5261		2.2371	

Table x. Fold-changes for BRB-E treated SCC-83 compared to control group.

		BRB-100		BRB-200		BRB-400	
Position	**Symbol**	**Fold Change**	**Comments**	**Fold Change**	**Comments**	**Fold Change**	**Comments**
A01	ACLY	0.8234	C	1.5367	C	0.4022	C
A02	ACSL4	1.266		1.1171		1.0683	
A03	ADM	0.6585		1.1305		0.7123	
A04	ANGPT1	0.594		1.3334		0.2324	
A05	ANGPT2	0.623		1.2887		0.3683	
A06	APAF1	1.0473		1.2294		0.9172	
A07	ARNT	0.717		1.1636		0.3559	
A08	ATP5A1	0.9193		1.0611		0.9702	
A09	AURKA	0.4467		1.0707		0.7444	
A10	BCL2L11	0.7316		1.057		0.5258	
A11	BIRC3	0.8912		1.2374		0.4628	
A12	BMI1	0.9139		1.359		0.9183	
B01	CA9	0.7232		1.4651		0.2734	
B02	CASP2	0.7954		0.95		0.6713	
B03	CASP7	1.0094		1.2498		0.8125	
B04	CASP9	0.7055		1.2066		0.4146	
B05	CCL2	0.5411		1.6779		0.3677	
B06	CCND2	0.4951		1.0707		0.2012	
B07	CCND3	0.7615		1.0198		0.757	
B08	CDC20	0.6406		0.8402		0.5731	
B09	CDH2	1.1404		1.1429		1.0191	
B10	CFLAR	1.2141		1.0069		0.7599	
B11	COX5A	1.0469		1.0978		0.8653	
B12	CPT2	0.7931		1.0132		0.4583	
C01	DDB2	0.8561		1.1717		0.7463	
C02	DDIT3	0.68		1.1526		0.4645	
C03	DKC1	0.9252		1.2512		1.0542	
C04	DSP	0.9695		1.1284		0.7988	
C05	E2F4	0.9453		0.9973		0.885	
C06	EPO	0.5701		1.3075		0.2193	
C07	ERCC3	1.0561		1.0944		0.9161	
C08	ERCC5	0.8046		1.0212		0.6297	
C09	ETS2	0.7075		1.1669		0.6048	
C10	FASLG	0.5204		1.1156		0.1863	
C11	FGF2	0.7268		0.7635		0.6473	
C12	FLT1	0.8309		1.4019		0.2275	
D01	FOXC2	1.1037		1.1369		0.785	
D02	G6PD	1.2246		1.2833		0.9921	
D03	GADD45G	0.4977		1.041		0.1622	
D04	GPD2	0.7648		1.1981		0.6529	
D05	GSC	0.5125		2.3989		0.2908	
D06	HMOX1	0.6182		0.8912		0.5311	
D07	IGFBP3	0.5855		1.2373		0.2507	
D08	IGFBP5	0.9799		1.1398		0.2028	
D09	IGFBP7	0.8117		1.2171		0.7294	
D10	KDR	0.5747		1.5065		0.1905	
D11	KRT14	0.5149		1.2592		0.2006	
D12	LDHA	1.2617		0.9954		1.2564	

E01	LIG4	0.7805		1.0498		0.5126	
E02	LPL	0.5338		1.3641		0.2038	
E03	MAP2K1	0.7151		0.9844		0.9159	
E04	MAP2K3	0.7102		0.7795		0.4663	
E05	MAPK14	1.023		1.0602		0.926	
E06	MCM2	1.3859		1.0308		1.0381	
E07	MKI67	1.0113		1.0185		0.7965	
E08	NOL3	0.7713		1.2164		0.594	
E09	OCLN	0.552		1.0476		0.2702	
E10	PFKL	0.9713		1.0106		0.8504	
E11	PGF	1.026		1.4317		0.9841	
E12	PINX1	0.6655		1.5616		0.6178	
F01	POLB	0.7487		1.1452		0.7669	
F02	PPP1R15A	0.7504		0.8529		0.6594	
F03	SERPINB2	0.7421		1.2149		0.4977	
F04	SERPINF1	0.714		1.1451		0.3269	
F05	SKP2	1.0222		0.9908		0.8446	
F06	SLC2A1	1.1028		0.9678		1.1009	
F07	SNAI1	0.5101		1.4859		0.2229	
F08	SNAI2	0.9855		1.182		1.0877	
F09	SNAI3	0.8247		1.0526		0.2867	
F10	SOD1	0.8341		0.9029		0.6391	
F11	SOX10	0.8663		1.6524		0.3119	
F12	STMN1	1.092		1.0178		0.9322	
G01	TBX2	0.692		1.4016		0.2581	
G02	TEK	0.5302		0.9784		0.1384	
G03	TEP1	1.001		1.1163		0.5591	
G04	TERF1	0.7794		1.0993		0.3671	
G05	TERF2IP	1.4139		1.2129		1.0229	
G06	TINF2	1.1948		1.1073		0.7998	
G07	TNKS	1.0453		1.1659		0.6811	
G08	TNKS2	1.3408		0.9079		0.9951	
G09	UQCRFS1	1.3037		1.0503		1.0506	
G10	VEGFC	1.002		0.9362		0.8234	
G11	WEE1	1.1684		0.8563		0.694	
G12	XIAP	1.3472		1.1353		0.9871	
H01	ACTB	1.4389		0.8578		1.2514	
H02	B2M	1.0175		1.1552		1.0269	
H03	GAPDH	1.0472		1.0031		1.0233	
H04	HPRT1	0.8071		0.9494		0.8307	
H05	RPLP0	1.1121		0.911		1.0876	
H06	HGDC	0.721		1.1389		0.1973	
H07	RTC	0.8234	C	1.5367	C	0.4022	C
H08	RTC	0.8234	C	1.5367	C	0.4022	C
H09	RTC	0.8234	C	1.5367	C	0.4022	C
H10	PPC	0.8836		1.7429		0.4505	
H11	PPC	0.8875		1.8675		0.4612	
H12	PPC	0.7827		1.6204		0.3591	

Table xi. Fold-changes for CS-E treated SCC-83 compared to control group.

		1.25% CS-E		2.5% CS-E		5% CS-E	
Position	**Symbol**	**Fold Change**	**Comments**	**Fold Change**	**Comments**	**Fold Change**	**Comments**
A01	ACLY	0.7271	C	0.9035	C	50.9537	C
A02	ACSL4	0.9889		1.1154		1.0508	
A03	ADM	0.9571		4.1483		5.1775	
A04	ANGPT1	0.5593		0.0911	A	36.7527	
A05	ANGPT2	1.4636	B	2.984	A	122.8402	B
A06	APAF1	0.7773		0.7775		3.7484	
A07	ARNT	1.0192		0.6763		14.8521	
A08	ATP5A1	0.8107		0.7614		0.8166	
A09	AURKA	0.8149		0.941		2.9948	
A10	BCL2L11	0.9401		1.1955		7.8174	
A11	BIRC3	0.81		0.5845		7.5851	
A12	BMI1	0.8981		1.1237		1.9128	
B01	CA9	2.8499	A	3.628	A	309.0038	A
B02	CASP2	0.853		0.9416		3.0107	
B03	CASP7	0.9438		0.9708		3.955	
B04	CASP9	0.7893		1.0314		11.9847	
B05	CCL2	2.633	B	0.9035	C	502.8627	B
B06	CCND2	0.6853	B	0.7605	B	81.0403	A
B07	CCND3	0.9414		1.4852		3.1944	
B08	CDC20	0.737		1.1516		3.1498	
B09	CDH2	0.8303		0.6998		1.7363	
B10	CFLAR	0.9597		0.7352		3.39	
B11	COX5A	0.8889		0.9088		1.354	
B12	CPT2	1.4842		1.3166		8.8557	
C01	DDB2	0.8487		0.6821		5.4224	
C02	DDIT3	1.8165		10.0985		27.4693	
C03	DKC1	0.7823		1.2247		2.4738	
C04	DSP	0.8612		0.783		4.3673	
C05	E2F4	0.7997		0.7945		1.4819	
C06	EPO	1.486	B	4.1173	B	2471.756	A
C07	ERCC3	23.8444		31.0916		103.9114	
C08	ERCC5	0.943		0.8218		5.631	
C09	ETS2	0.7024		0.9428		2.8901	
C10	FASLG	2.0966	B	0.4184	B	858.2455	A
C11	FGF2	0.863		0.7293		4.6709	
C12	FLT1	0.6644	B	0.304	B	533.7434	A
D01	FOXC2	0.9614		1.1366		9.2932	
D02	G6PD	1.0764		0.8911		0.342	
D03	GADD45G	0.4755	B	0.8888	B	583.0839	A
D04	GPD2	0.8816		0.7447		2.592	
D05	GSC	0.7271	C	0.9035	C	837.3034	B
D06	HMOX1	1.1793		4.728		3.1091	
D07	IGFBP3	0.9185		1.3863		33.062	
D08	IGFBP5	0.4638		0.6257		19.453	
D09	IGFBP7	0.8543		0.6139		0.7649	
D10	KDR	0.7271	C	2.0918	B	1173.5777	B
D11	KRT14	3.173	B	2.0846	B	1799.7286	A
D12	LDHA	0.8704		0.8318		0.9501	

E01	LIG4	0.8714		1.2256		5.5055	
E02	LPL	1.9844	B	4.635	B	1358.1213	B
E03	MAP2K1	1.0154		1.4064		2.3421	
E04	MAP2K3	1.0508		2.7081		12.2677	
E05	MAPK14	0.9393		0.7585		3.4824	
E06	MCM2	0.942		0.9426		3.6582	
E07	MKI67	0.8392		0.7237		2.0039	
E08	NOL3	0.7709		0.7162		8.6798	
E09	OCLN	1.3735		1.4981		52.386	
E10	PFKL	1.0101		0.4967		0.9973	
E11	PGF	0.7669		2.538		2.7361	
E12	PINX1	0.8028		1.5569		6.6941	A
F01	POLB	0.705		1.0271		0.0769	A
F02	PPP1R15A	0.5862		19.0001		13.6287	
F03	SERPINB2	5.7275		81.395		37.7811	A
F04	SERPINF1	0.7925		1.0457		30.2877	
F05	SKP2	0.8904		0.6618		3.2103	
F06	SLC2A1	0.8334		0.8303		1.5274	
F07	SNAI1	5.4851	B	547.5616	A	400.7875	B
F08	SNAI2	1.2556		1.6193		8.0657	
F09	SNAI3	0.7613		0.8764		48.7376	
F10	SOD1	0.9194		1.3132		2.3139	
F11	SOX10	0.5923	B	0.9629	B	507.3775	A
F12	STMN1	0.7459		0.7238		1.138	
G01	TBX2	0.8262		1.6526		1.5708	A
G02	TEK	1.5335	B	1.4852	B	682.4056	A
G03	TEP1	0.8985		0.6972		7.1684	
G04	TERF1	0.4681		0.4862		30.2482	
G05	TERF2IP	0.9058		1.41		1.7921	
G06	TINF2	1.1449		1.1346		4.7544	
G07	TNKS	0.9818		0.7974		3.4993	
G08	TNKS2	1.0233		0.501		1.6483	
G09	UQCRFS1	1.0722		1.1075		1.387	
G10	VEGFC	1.1132		1.3104		1.4214	A
G11	WEE1	0.8334		0.8325		2.218	
G12	XIAP	0.8171		0.6156		1.6303	
H01	ACTB	0.7696		1.1877		0.0002	A
H02	B2M	1.3298		0.8447		0.6075	
H03	GAPDH	0.8194		0.9863		0.9836	
H04	HPRT1	0.9167		1.2059		1.8352	
H05	RPLP0	0.9866		1.0309		1.0781	
H06	HGDC	2.4548	B	2.7184	B	2141.7608	A
H07	RTC	0.7271	C	0.9035	C	50.9537	C
H08	RTC	0.7271	C	0.9035	C	50.9537	C
H09	RTC	0.7271	C	0.9035	C	50.9537	C
H10	PPC	0.5911		0.723		52.6254	
H11	PPC	0.5882		0.7302		59.8066	
H12	PPC	0.5473		0.6416		48.9759	

Table xii. Fold-changes for BRB-E treated CA-83 compared to control group.

		BRB-100		BRB-200		BRB-400	
Position	**Symbol**	**Fold Change**	**Comments**	**Fold Change**	**Comments**	**Fold Change**	**Comments**
A01	ACLY	1.0093	C	1.2665	C	0.8971	C
A02	ACSL4	0.9161		0.0192		0.018	
A03	ADM	3.5203		0.0978		0.0639	
A04	ANGPT1	0.1323	A	1.1877		1.0948	
A05	ANGPT2	1.656	A	1.5168	B	2.079	A
A06	APAF1	0.8124		0.0871		0.1144	
A07	ARNT	0.539		0.2633		0.4574	
A08	ATP5A1	0.7629		0.0182		0.0155	
A09	AURKA	0.9379		0.0664		0.0564	
A10	BCL2L11	1.0329		0.1503		0.1249	
A11	BIRC3	0.5861		0.1692		0.203	
A12	BMI1	1.0163		0.0385		0.0516	
B01	CA9	1.034		1.9595		2.3903	
B02	CASP2	0.8966		0.0638		0.0448	
B03	CASP7	0.8354		0.0757		0.0469	
B04	CASP9	1.0613		0.2744		0.356	
B05	CCL2	0.2787	B	3.4515	B	5.4386	B
B06	CCND2	0.9013	B	2.137	A	3.6565	A
B07	CCND3	1.2814		16.1187		11.8335	
B08	CDC20	1.2691		3.2565		2.5153	
B09	CDH2	0.6845		3.869		2.4379	
B10	CFLAR	0.6222		0.012	A	0.0273	A
B11	COX5A	0.8304		0.005		0.0056	
B12	CPT2	0.7205		4.0942		3.2087	
C01	DDB2	1.9035		1.7246		1.3907	
C02	DDIT3	0.9623		1.2826		0.9708	
C03	DKC1	1.0686		1.2818		5.3267	
C04	DSP	0.9603		1.272		0.9095	
C05	E2F4	1.1678		1.4324		0.8729	
C06	EPO	1.098		1.3928		0.8929	
C07	ERCC3	1.0977		0.0028		0.002	
C08	ERCC5	1.0088		0.3965		0.3517	
C09	ETS2	0.9912		0.2989		0.2157	
C10	FASLG	1.0549	B	39.0366	A	23.5558	A
C11	FGF2	1.1757		0.123	A	0.1398	A
C12	FLT1	1.2157		0.0317		0.0114	A
D01	FOXC2	0.9736		1.2564		1.0644	
D02	G6PD	1.0715		1.3544		1.0819	
D03	GADD45G	0.9415		1.1477		0.784	
D04	GPD2	1.2069		1.4229		1.0571	
D05	GSC	0.9374		1.2075		0.9232	
D06	HMOX1	1.4339		1.3717		1.2093	
D07	IGFBP3	0.9125		31.291		25.3172	
D08	IGFBP5	0.9094		0.1598		0.1214	
D09	IGFBP7	1.0958		2.7109		2.1044	
D10	KDR	1.0254		2.5301		1.9919	
D11	KRT14	4.1697	B	4171.9985	A	2948.0156	A
D12	LDHA	1.9759	A	1.3777	B	1.4988	A

E01	LIG4	1.0135		1.3265		1.0332	
E02	LPL	1.1475		1.2899		0.9102	
E03	MAP2K1	1.1089		1.3806		1.0265	
E04	MAP2K3	2.379	B	2.1626	B	0.9971	B
E05	MAPK14	1.1909		1.4965		0.9561	
E06	MCM2	1.8203	B	1.6067	B	1.988	B
E07	MKI67	1.4406		1.8908		1.3366	
E08	NOL3	1.4085		19.3987		19.2644	
E09	OCLN	1.1373		0.0061	A	0.0067	A
E10	PFKL	0.9293		0.7787		0.6007	
E11	PGF	0.9859		0.0016	A	0.0011	A
E12	PINX1	1.5139	B	107.6481	A	75.7727	A
F01	POLB	0.9767		0.9857		0.8499	
F02	PPP1R15A	4.273		5.0108		1.7838	
F03	SERPINB2	1.1258		1.3783		0.6823	
F04	SERPINF1	1.6267	B	0.1896	B	1.195	B
F05	SKP2	1.4705		0.7404	A	1.5774	
F06	SLC2A1	1.2249		1.5085		1.2805	
F07	SNAI1	1.0099		0.4903		0.4395	
F08	SNAI2	1.2908		0.0018	A	0.0027	A
F09	SNAI3	1.4764	A	185.5866	A	131.4596	A
F10	SOD1	1.093		1.0209		0.7218	
F11	SOX10	10.8657	B	1200.1532	A	914.5318	A
F12	STMN1	0.9609		4.7453		4.1441	
G01	TBX2	1.1425		1.5394		1.0182	
G02	TEK	0.9225		1.3015		0.9711	
G03	TEP1	1.299		1.357		1.591	
G04	TERF1	0.9814		1.1071		1.054	
G05	TERF2IP	1.0803		1.4748		1.3642	
G06	TINF2	2.501		1.1122		0.9599	
G07	TNKS	1.1144		2.1812		1.5601	
G08	TNKS2	1.5489	B	320.8241	A	245.8592	A
G09	UQCRFS1	1.2758		0.0427		0.0533	
G10	VEGFC	1.0845		0.4256		0.2944	
G11	WEE1	1.1747		4.2536		2.8597	
G12	XIAP	1.1549		1.6136		1.235	
H01	ACTB	1.3103	B	1.3261	B	1.179	B
H02	B2M	1.1162		1.2815		1.7394	
H03	GAPDH	0.9358		1.2813		1.2788	
H04	HPRT1	0.999		1.2741		0.9087	
H05	RPLP0	0.9411		0.4096	A	0.4424	A
H06	HGDC	1.1459		1.4026		0.9659	
H07	RTC	1.1061		0.1174		0.0863	
H08	RTC	1.0014		0.0149		0.0107	
H09	RTC	0.9676		3.4734		2.1963	
H10	PPC	1.1527		2.6359		1.6868	
H11	PPC	1.1772		0.6857		0.5873	
H12	PPC	1.0253		0.3026		0.2207	

Table xiii. Fold-changes for CS-E treated CA-83 compared to control group.

		1.25% CS-E		2.5% CS-E		5% CS-E	
Position	**Symbol**	**Fold Change**	**Comments**	**Fold Change**	**Comments**	**Fold Change**	**Comments**
A01	ACLY	1.269	C	3.0257	C	28.1292	C
A02	ACSL4	0.789		0.9104		0.3592	
A03	ADM	5.1561		14.152		4.2192	
A04	ANGPT1	1.929	A	0.2225	B	38.0224	B
A05	ANGPT2	0.9819	B	1.61	B	5.7455	B
A06	APAF1	0.5906		0.7733		1.3793	
A07	ARNT	0.958		0.4297		1.8162	A
A08	ATP5A1	0.6629		0.4602		0.5059	
A09	AURKA	0.6149		0.7		1.4039	
A10	BCL2L11	1.6967		3.9427		4.947	
A11	BIRC3	0.6225		0.1868		0.7808	
A12	BMI1	0.9237		1.147		0.6365	
B01	CA9	25.1124	A	56.6008	A	302.9215	A
B02	CASP2	0.8081		0.8005		1.038	
B03	CASP7	1.0033		1.0699		1.7283	
B04	CASP9	0.8733		0.7865		1.4265	
B05	CCL2	4.8868	B	1.8581	B	208.0794	B
B06	CCND2	1.915	B	3.9152	B	86.374	B
B07	CCND3	0.9677		1.4069		1.857	
B08	CDC20	0.5798		0.828		1.4674	
B09	CDH2	0.7375		0.574		0.6222	
B10	CFLAR	0.8328		0.4534		1.495	
B11	COX5A	0.8858		0.8387		1.1678	
B12	CPT2	0.9059		0.2472		1.539	
C01	DDB2	0.7261		0.6512		1.5096	
C02	DDIT3	8.4113		23.9531		8.1318	
C03	DKC1	0.7257		1.0944		1.0106	
C04	DSP	0.8976		0.7102		0.5	A
C05	E2F4	0.8191		0.8594		1.2808	
C06	EPO	1.8406	B	1.5381	B	115.0162	B
C07	ERCC3	0.6682		0.7977		0.9507	
C08	ERCC5	0.7439		0.5458		1.3522	
C09	ETS2	1.2612		3.7115		1.605	
C10	FASLG	2.3146	B	0.4497	B	77.5504	B
C11	FGF2	0.8905		0.9203		1.9089	
C12	FLT1	24.6008	B	26.1276	B	1074.7847	A
D01	FOXC2	0.7437		0.933		3.0066	
D02	G6PD	0.7842		0.4341		0.4106	
D03	GADD45G	13.9843	A	8.4942	B	351.9742	A
D04	GPD2	0.7307		0.5885		0.9783	
D05	GSC	6.7933	B	10.7173	B	337.1178	B
D06	HMOX1	4.2275		10.8023		1.0935	
D07	IGFBP3	2.7574		5.9489		7.2688	
D08	IGFBP5	0.7353		0.4559		1.4479	
D09	IGFBP7	0.9886		0.5491		0.7373	
D10	KDR	13.8498	B	3.0257	C	722.3975	B
D11	KRT14	6.1573	A	4.2424	B	102.2128	A
D12	LDHA	0.6651		0.509		0.52	

E01	LIG4	0.8302		1.2146		1.1887	
E02	LPL	11.121	B	12.183	B	449.7739	B
E03	MAP2K1	0.8364		1.1794		0.9007	
E04	MAP2K3	1.8754		6.0359		2.9764	
E05	MAPK14	0.7112		0.5295		1.1797	
E06	MCM2	0.623		0.7365		0.6584	
E07	MKI67	0.4015		0.3878		0.8829	
E08	NOL3	0.9742		0.591		1.9471	
E09	OCLN	0.8132		0.4304		5.387	
E10	PFKL	0.8909		0.5443		0.6189	
E11	PGF	1.6775		6.1944		1.2062	
E12	PINX1	0.8587		0.8895		2.2771	
F01	POLB	0.9978		1.1783		1.7028	
F02	PPP1R15A	7.1932		39.5301		5.4411	
F03	SERPINB2	3.0295		20.7848		11.383	A
F04	SERPINF1	0.98		0.9125		4.5117	
F05	SKP2	0.6207		0.3885		0.904	
F06	SLC2A1	0.8483		0.535		0.7533	
F07	SNAI1	530.3012	A	2176.5056	A	154.6432	A
F08	SNAI2	1.0539		1.1198		1.6815	
F09	SNAI3	1.5143		1.0629	A	4.2814	A
F10	SOD1	1.0778		1.7819		1.1824	
F11	SOX10	2.9612	A	1.2169	B	41.8393	B
F12	STMN1	0.7435		1.0878		1.3283	
G01	TBX2	0.9895		2.998		14.9383	
G02	TEK	46.9019	A	27.7323	B	2458.2774	A
G03	TEP1	1.5366		1.1161		1.7696	
G04	TERF1	1.2101		1.3209		8.6504	
G05	TERF2IP	1.1402		1.051		1.1371	
G06	TINF2	0.9141		1.1308		1.5129	
G07	TNKS	0.7382		0.5555		0.7086	A
G08	TNKS2	0.7054		0.6117		0.8847	
G09	UQCRFS1	0.7747		0.5627		0.7276	
G10	VEGFC	0.9049		0.621		0.849	
G11	WEE1	0.8533		1.2685		1.1921	
G12	XIAP	0.7972		0.4715		0.6878	
H01	ACTB	0.9901		1.2143		1.2326	
H02	B2M	1.261		0.7556		1.1081	
H03	GAPDH	0.9141		1.0133		0.9784	
H04	HPRT1	0.9135		1.2693		1.2358	
H05	RPLP0	0.9463		1.0944		0.8303	
H06	HGDC	11.7062	B	14.8558	B	395.2537	B
H07	RTC	1.269	C	3.0257	C	28.1292	C
H08	RTC	1.269	C	3.0257	C	28.1292	C
H09	RTC	1.269	C	3.0257	C	28.1292	C
H10	PPC	1.2664		3.3529		25.0095	
H11	PPC	1.1003		3.2043		24.5258	
H12	PPC	0.6977		3.0067		27.0043	

Table xiv. Interpretation of comments.

Comment
A: This gene's average threshold cycle is relatively high (> 30) in either the control or the test sample, and is reasonably low in the other sample (< 30). These data mean that the gene's expression is relatively low in one sample and reasonably detected in the other sample suggesting that the actual fold-change value is at least as large as the calculated and reported fold-change result. This fold-change result may also have greater variations if p value > 0.05; therefore, it is important to have a sufficient number of biological replicates to validate the result for this gene.
B: This gene's average threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and test samples, and the p-value for the fold-change is either unavailable or relatively high (p > 0.05). This fold-change result may also have greater variations; therefore, it is important to have a sufficient number of biological replicates to validate the result for this gene.
C: This gene's average threshold cycle is either not determined or greater than the defined cut-off value (default 35), in both samples meaning that its expression was undetected, making this fold-change result erroneous and un-interpretable.

Table xv. Genes tested in Human Cancer PathwayFinder PCR Array (Cat. No.) PAHS-033Z

Unigene	Refseq	Symbol	Description	Gname	RT2 Catalog
Hs.387567	NM_001096	ACLY	ATP citrate lyase	ACL/ATPCL/CLATP	PPH00021A
Hs.268785	NM_004458	ACSL4	Acyl-CoA synthetase long-chain family member 4	ACS4/FACL4/LACS4/MRX63/MRX68	PPH15399A
Hs.441047	NM_001124	ADM	Adrenomedullin	AM/PAMP	PPH02039C
Hs.369675	NM_001146	ANGPT1	Angiopoietin 1	AGP1/AGPT/ANG1	PPH00374B
Hs.583870	NM_001147	ANGPT2	Angiopoietin 2	AGPT2/ANG2	PPH00377F
Hs.552567	NM_001160	APAF1	Apoptotic peptidase activating factor 1	APAF-1/CED4	PPH00752A
Hs.632446	NM_001668	ARNT	Aryl hydrocarbon receptor nuclear translocator	HIF-1-beta/HIF-1beta/HIF1-beta/HIF1B/HIF1BETA/TANGO/bHLHe2	PPH01233B
Hs.298280	NM_004046	ATP5A1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle	ATP5A/ATP5AL2/ATPM/COXPD22/HEL-S-123m/MC5DN4/MOM2/OMR/ORM/hATP1	PPH02335A
Hs.250822	NM_003600	AURKA	Aurora kinase A	AIK/ARK1/AURA/AURORA2/BTAK/PPP1R47/STK15/STK6/STK7	PPH15095A
Hs.469658	NM_006538	BCL2L11	BCL2-like 11 (apoptosis facilitator)	BAM/BIM/BOD	PPH00893F
Hs.127799	NM_001165	BIRC3	Baculoviral IAP repeat containing 3	AIP1/API2/CIAP2/HAIP1/HIAP1/MALT2/MIHC/RNF49/c-IAP2	PPH00326B
Hs.380403	NM_005180	BMI1	BMI1 polycomb ring finger oncogene	FLVI2/BMI1/PCGF4/RNF51/flvi-2/bmi-1	PPH57778A
Hs.63287	NM_001216	CA9	Carbonic anhydrase IX	CAIX/MN	PPH01751A
Hs.368982	NM_032982	CASP2	Caspase 2, apoptosis-related cysteine peptidase	CASP-2/ICH1/NEDD-2/NEDD2/PPP1R57	PPH00111A
Hs.9216	NM_001227	CASP7	Caspase 7, apoptosis-related cysteine peptidase	CASP-7/CMH-1/ICE-LAP3/LICE2/MCH3	PPH00110C

Hs.329502	NM_001229	CASP9	Caspase 9, apoptosis-related cysteine peptidase	APAF-3/APAF3/ICE-LAP6/MCH6/PPP1R56	PPH00353B
Hs.303649	NM_002982	CCL2	Chemokine (C-C motif) ligand 2	GDCF-2/HC11/HSMCR30/MCAF/MCP-1/MCP1/SCYA2/SMC-CF	PPH00192F
Hs.376071	NM_001759	CCND2	Cyclin D2	KIAK0002/MPPH3	PPH00129C
Hs.534307	NM_001760	CCND3	Cyclin D3	-	PPH00130C
Hs.524947	NM_001255	CDC20	Cell division cycle 20 homolog (S. cerevisiae)	CDC20A/bA276H19.3/p55CDC	PPH00306F
Hs.464829	NM_001792	CDH2	Cadherin 2, type 1, N-cadherin (neuronal)	CD325/CDHN/CDw325/NCAD	PPH00636F
Hs.390736	NM_003879	CFLAR	CASP8 and FADD-like apoptosis regulator	CASH/CASP8AP1/CLARP/Casper/FLAME/FLAME-1/FLAME1/FLIP/I-FLICE/MRIT/c-FLIP/c-FLIPL/c-FLIPR/c-FLIPS	PPH00333B
Hs.401903	NM_004255	COX5A	Cytochrome c oxidase subunit Va	COX/COX-VA/VA	PPH19106C
Hs.713535	NM_000098	CPT2	Carnitine palmitoyltransferase 2	CPT1/CPTASE/IIAE4	PPH15572A
Hs.700338	NM_000107	DDB2	Damage-specific DNA binding protein 2, 48kDa	DDBB/UV-DDB2	PPH01726A
Hs.505777	NM_004083	DDIT3	DNA-damage-inducible transcript 3	CEBPZ/CHOP/CHOP-10/CHOP10/GADD153	PPH00310A
Hs.4747	NM_001363	DKC1	Dyskeratosis congenita 1, dyskerin	CBF5/DKC/DKCX/NAP57/NOLA4/XAP101	PPH07260G
Hs.519873	NM_004415	DSP	Desmoplakin	DCWHKTA/DP/DPI/DPII	PPH17443A
Hs.108371	NM_001950	E2F4	E2F transcription factor 4, p107/p130-binding	E2F-4	PPH00362F
Hs.2303	NM_000799	EPO	Erythropoietin	EP/MVCD2	PPH01338C
Hs.469872	NM_000122	ERCC3	Excision repair cross-complementing rodent repair deficiency, complementation group 3 (xeroderma pigmentosum group B complementing)	BTF2/GTF2H/RAD25/TFIIH/TTD2/XPB	PPH01543A
Hs.258429	NM_000123	ERCC5	Excision repair cross-complementing	COFS3/ERCC5-201/ERCM2/UVDR/XPG/XPGC	PPH01538A

			rodent repair deficiency, complementation group 5		
Hs.644231	NM_005239	ETS2	V-Ets erythroblastosis virus E26 oncogene homolog 2 (avian)	ETS2IT1	PPH00091C
Hs.2007	NM_000639	FASLG	Fas ligand (TNF superfamily, member 6)	ALPS1B/APT1LG1/APTL/CD178/CD95-L/CD95L/FASL/TNFSF6	PPH00142C
Hs.284244	NM_002006	FGF2	Fibroblast growth factor 2 (basic)	BFGF/FGF-2/FGFB/HBGF-2	PPH00257C
Hs.594454	NM_002019	FLT1	Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	FLT/FLT-1/VEGFR-1/VEGFR1	PPH00375D
Hs.436448	NM_005251	FOXC2	Forkhead box C2 (MFH-1, mesenchyme forkhead 1)	FKHL14/LD/MFH-1/MFH1	PPH01971A
Hs.461047	NM_000402	G6PD	Glucose-6-phosphate dehydrogenase	G6PD1	PPH02322H
Hs.9701	NM_006705	GADD45G	Growth arrest and DNA-damage-inducible, gamma	CR6/DDIT2/GADD45gamma/GRP17	PPH02207A
Hs.512382	NM_000408	GPD2	Glycerol-3-phosphate dehydrogenase 2 (mitochondrial)	GDH2/GPDM/mGPDH	PPH11093C
Hs.440438	NM_173849	GSC	Goosecoid homeobox	SAMS	PPH01920A
Hs.727017	NM_002133	HMOX1	Heme oxygenase (decycling) 1	HMOX1D/HO-1/HSP32/bK286B10	PPH00161F
Hs.450230	NM_000598	IGFBP3	Insulin-like growth factor binding protein 3	BP-53/IBP3	PPH00281C
Hs.607212	NM_000599	IGFBP5	Insulin-like growth factor binding protein 5	IBP5	PPH00312C
Hs.691061	NM_001553	IGFBP7	Insulin-like growth factor binding protein 7	AGM/FSTL2/IBP-7/IGFBP-7/IGFBP-7v/IGFBPRP1/MAC25/PSF/RAMSVPS/TAF	PPH05831G
Hs.479756	NM_002253	KDR	Kinase insert domain receptor (a type III receptor tyrosine kinase)	CD309/FLK1/VEGFR/VEGFR2	PPH00386A
Hs.654380	NM_000526	KRT14	Keratin 14	CK14/EBS3/EBS4/K14/NFJ	PPH02389A
Hs.2795	NM_005566	LDHA	Lactate dehydrogenase A	GSD11/HEL-S-133P/LDHM/PIG19	PPH02047H

Hs.166091	NM_002312	LIG4	Ligase IV, DNA, ATP-dependent	LIG4S	PPH00746F
Hs.180878	NM_000237	LPL	Lipoprotein lipase	HDLCQ11/LIPD	PPH00023C
Hs.145442	NM_002755	MAP2K1	Mitogen-activated protein kinase kinase 1	CFC3/MAPKK1/MEK1/MKK1/PRKMK1	PPH00711C
Hs.514012	NM_002756	MAP2K3	Mitogen-activated protein kinase kinase 3	MAPKK3/MEK3/MKK3/PRKMK3/SAPKK-2/SAPKK2	PPH00747F
Hs.485233	NM_001315	MAPK14	Mitogen-activated protein kinase 14	CSBP/CSBP1/CSBP2/CSPB1/EXIP/Mxi2/PRKM14/PRKM15/RK/SAPK2A/p38/p38ALPHA	PPH00750B
Hs.477481	NM_004526	MCM2	Minichromosome maintenance complex component 2	BM28/CCNL1/CDCL1/D3S3194/MITOTIN/cdc19	PPH00961A
Hs.689823	NM_002417	MKI67	Antigen identified by monoclonal antibody Ki-67	KIA/MIB-/MIB-1/PPP1R105	PPH01024E
Hs.513667	NM_003946	NOL3	Nucleolar protein 3 (apoptosis repressor with CARD domain)	ARC/FCM/MYP/NOP/NOP30	PPH00902F
Hs.592605	NM_002538	OCLN	Occludin	BLCPMG/PPP1R115	PPH02571B
Hs.255093	NM_002626	PFKL	Phosphofructokinase, liver	ATP-PFK/PFK-B/PFK-L	PPH02048A
Hs.252820	NM_002632	PGF	Placental growth factor	D12S1900/PGFL/PLGF/PlGF-2/SHGC-10760	PPH01155F
Hs.490991	NM_017884	PINX1	PIN2/TERF1 interacting, telomerase inhibitor 1	LPTL/LPTS	PPH222944B
Hs.661106	NM_002690	POLB	Polymerase (DNA directed), beta	-	PPH137355F
Hs.631593	NM_014330	PPP1R15A	Protein phosphatase 1, regulatory (inhibitor) subunit 15A	GADD34	PPH02081E
Hs.594481	NM_002575	SERPINB2	Serpin peptidase inhibitor, clade B (ovalbumin), member 2	HsT1201/PAI/PAI-2/PAI2/PLANH2	PPH00793C
Hs.532768	NM_002615	SERPINF1	Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	EPC-1/OI12/OI6/PEDF/PIG35	PPH00805A
Hs.23348	NM_005983	SKP2	S-phase kinase-associated protein 2 (p45)	FBL1/FBXL1/FLB1/p45	PPH00232B

Hs.473721	NM_006516	SLC2A1	Solute carrier family 2 (facilitated glucose transporter), member 1	CSE/DYT17/DYT18/DYT9/EIG12/GLUT/GLUT-1/GLUT1/GLUT1DS/HTLVR/PED	PPH02043C
Hs.48029	NM_005985	SNAI1	Snail homolog 1 (Drosophila)	SLUGH2/SNA/SNAH/SNAIL/SNAIL1/dJ710H13.1	PPH02459B
Hs.360174	NM_003068	SNAI2	Snail homolog 2 (Drosophila)	SLUG/SLUGH1/SNAIL2/WS2D	PPH02475A
Hs.673548	NM_178310	SNAI3	Snail homolog 3 (Drosophila)	SMUC/SNAIL3/ZNF293/Zfp293	PPH15155C
Hs.443914	NM_000454	SOD1	Superoxide dismutase 1, soluble	ALS/ALS1/HEL-S-44/IPOA/SOD/hSod1/homodimer	PPH00234B
Hs.376984	NM_006941	SOX10	SRY (sex determining region Y)-box 10	DOM/PCWH/WS2E/WS4/WS4C	PPH02458C
Hs.209983	NM_005563	STMN1	Stathmin 1	C1orf215/LAP18/Lag/OP18/PP17/PP19/PR22/SMN	PPH14448B
Hs.531085	NM_005994	TBX2	T-box 2	-	PPH13373C
Hs.89640	NM_000459	TEK	TEK tyrosine kinase, endothelial	CD202B/TIE-2/TIE2/VMCM/VMCM1	PPH00795B
Hs.508835	NM_007110	TEP1	Telomerase-associated protein 1	TLP1/TP1/TROVE1/VAULT2/p240	PPH02434A
Hs.442707	NM_017489	TERF1	Telomeric repeat binding factor (NIMA-interacting) 1	PIN2/TRBF1/TRF/TRF1/hTRF1-AS/t-TRF1	PPH02426A
Hs.301419	NM_018975	TERF2IP	Telomeric repeat binding factor 2, interacting protein	DRIP5/RAP1	PPH15589C
Hs.496191	NM_012461	TINF2	TERF1 (TRF1)-interacting nuclear factor 2	DKCA3/TIN2	PPH02468A
Hs.370267	NM_003747	TNKS	Tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase	ARTD5/PARP-5a/PARP5A/PARPL/TIN1/TINF1/TNKS1/pART5	PPH19448A
Hs.329327	NM_025235	TNKS2	Tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase 2	ARTD6/PARP-5b/PARP-5c/PARP5B/PARP5C/TANK2/TNKL/pART6	PPH15823A
Hs.743307	NM_006003	UQCRFS1	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	RIP1/RIS1/RISP/UQCR5	PPH17737A
Hs.435215	NM_005429	VEGFC	Vascular endothelial growth factor C	Flt4-L/LMPH1D/VRP	PPH00673D
Hs.249441	NM_003390	WEE1	WEE1 homolog (S. pombe)	WEE1A/WEE1hu	PPH00445A

Hs.356076	NM_001167	XIAP	X-linked inhibitor of apoptosis	API3/BIRC4/IAP-3/ILP1/MIHA/XLP2/hIAP-3/hIAP3	PPH00323A
Hs.520640	NM_001101	ACTB	Actin, beta	BRWS1/PS1TP5BP1	PPH00073G
Hs.534255	NM_004048	B2M	Beta-2-microglobulin	-	PPH01094E
Hs.592355	NM_002046	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	G3PD/GAPD/HEL-S-162eP	PPH00150F
Hs.412707	NM_000194	HPRT1	Hypoxanthine phosphoribosyltransferase 1	HGPRT/HPRT	PPH01018C
Hs.546285	NM_001002	RPLP0	Ribosomal protein, large, P0	L10E/LP0/P0/PRLP0/RPP0	PPH21138F
N/A	SA_00105	HGDC	Human Genomic DNA Contamination	HIGX1A	
N/A	SA_00104	RTC	Reverse Transcription Control	RTC	PPX63340A
N/A	SA_00104	RTC	Reverse Transcription Control	RTC	PPX63340A
N/A	SA_00104	RTC	Reverse Transcription Control	RTC	PPX63340A
N/A	SA_00103	PPC	Positive PCR Control	PPC	
N/A	SA_00103	PPC	Positive PCR Control	PPC	
N/A	SA_00103	PPC	Positive PCR Control	PPC	